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Refsum's Disease

Transferrin and the
Metabolism of Iron

The Pediatric Assistant:
A New Role in the Child
Health Care Crisis

Book Review

Abstracts

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Address communications:
Editor
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HEREDOPATHIA ATACTICA POLYNEURITIFORMIS— AN INBORN ERROR OF LIPID METABOLISM INVOLVING THE NERVOUS SYSTEM: SOME RECENT BIOCHEMICAL AND DIETARY STUDIES

Sigvald Refsum

The clinical picture of heredopathia atactica polyneuritiformis, which I first described in 1944 and 1945,[1] is characterized by the following manifestations when fully developed: tapeto-retinal degeneration (retinitis pigmentosa); chronic polyneuropathy; ataxia and other cerebellar signs; and increased cerebrospinal fluid protein with a normal cell count. In most cases the following findings are also present: neurogenic (cochlear) impairment of hearing and electrocardiographic changes. In some cases pupillary abnormalities, lens opacities, anosmia, skeletal malformations, and skin changes (sometimes resembling ichthyosis) are encountered. The age at onset has varied from early childhood[2] to the second and third decades. As a rule, the onset is insidious, apparently often precipitated by infections. The course is often relapsing, with an overall tendency to progression. Sudden death has occurred in several cases.

The disease has been reported in some 50 patients from approximately 30 families in several countries. Males and females are affected in equal numbers, and it is probably caused by an autosomal recessive gene.[1,2,3]

Recent reviews of heredopathia atactica polyneuritiformis have been published by Kahlke[4] and Steinberg, *et al.*[5]

Neuropathological examinations were first described by Cammermeyer[6] in 1946, 1949, and 1956. Demyelination and interstitial hypertrophic polyneuropathy have been encountered in the peripheral nerves. In the spinal cord, atrophy of the posterior columns is a fairly constant finding. The anterior horn motor cells are reduced in number, with others showing signs of degeneration. Patchy myelin loss has been found in the brain stem. Nerve cell loss and degenerative changes have been observed in the olivary, vestibular, cochlear, vagal, dentate, and red nuclei. Lipid cells and gliosis are found in the damaged areas.

In 1963 Klenk and Kahlke reported high concentrations of a branched-chain fatty acid, 3,7,11,15-tetramethyl hexadecanoic or phytanic acid, in the liver and kidney of a young girl who died from heredopathia atactica polyneuritiformis.[7] This fatty acid, previously unobserved in man, was later also found in high concentrations in the serum from patients with this disease. It has now been demonstrated in 27 typical cases.[8] The accumulation of phytanic acid appears to be specific for heredopathia atactica polyneuritiformis. On the other hand, elevated serum phytanic acid has never

From the Department of Neurology, Division of Medicine, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Sigvald Refsum, M.D., Professor and Chairman, Department of Neurology, University of Oslo, Rikshospitalet; Visiting Professor, Department of Neurology, Presbyterian-St. Luke's Hospital

been found in any other disease. Normal serum phytanic acid levels have been reported in 19 patients with hypertrophic interstitial neuropathy of the Dejerine-Sottas type. However, phytanic acid accumulation has been demonstrated in a few patients who fulfilled most of the clinical criteria of heredopathia atactica polyneuritiformis. I personally feel that the diagnosis in these cases may be somewhat questionable.

Experiments have demonstrated that no endogenous synthesis of phytanic acid takes place. The likely source is dietary phytol (which is a major constituent of the chlorophyll molecule) or preformed phytanic acid in animal fat and butter. Eldjarn, in 1965, postulated that the accumulation might be caused by a defect in the breakdown mechanism of phytanic acid. Such a defect would be in accord with what is known of other inborn errors of metabolism where the defect usually may be traced back to the absence of an enzyme, leading to an accumulation of the products of the preceding steps in the reaction.

Independent studies in three laboratories have shown that patients with heredopathia atactica polyneuritiformis have a defect in the oxidative pathway for degradation of phytanic acid. This metabolic defect, which is only partial, appears to exist in one of the initial enzymatic reactions of the alpha-oxidation mechanism of phytanic acid (Avigan, *et al.*,[9] Eldjarn, *et al.*,[10] Shorland, *et al.*,[11] Mize, *et al.*,[12] Steinberg, *et al.*,[13,14] Stokke, *et al.*[15]). Recent studies of the metabolism of phytanic acid in tissue cultures derived from homozygotes and heterozygotes are of great interest (Herndon, *et al.*,[16,17] Mize, *et al.*[18]). It has been demonstrated that fibroblasts derived from skin biopsies of 14 patients with heredopathia atactica polyneuritiformis show greatly depressed rates of phytanic acid oxidation. On the average, the observed rate of oxidation was three per cent of that observed in cultures derived from control subjects. These studies strengthen the proposal that patients with heredopathia atactica polyneuritiformis accumulate phytanic acid because of a block in its degradation at the initial alpha-oxidative step.

Cultures derived from eight presumed heterozygotes, parents of the patients, oxidized phytanic acid at a rate approximately one-half that observed in control cultures. The finding of a partial defect in ability to oxidize phytanic acid in cultures derived from heterozygous parents represents the first demonstration of a carrier state in heredopathia atactica polyneuritiformis. The results of these studies of fibroblast cultures strongly support the thesis that the disorder is inherited in an autosomal recessive pattern.

When the experimental studies indicated that the phytanic acid was of exogenous origin, we began in 1965 a dietary treatment of two Norwegian patients with heredopathia atactica polyneuritiformis. It consists of a closely controlled restriction in the dietary intake of foods containing phytanic acid and phytol (Eldjarn, *et al.*[19]). Such a diet has now been employed for more than four and one-half years and has resulted in a marked fall of the serum phytanic acid level. During this treatment, there has been subjective and objective clinical improvement. The nerve conduction velocity has increased, the electrocardiographic findings have improved, and the protein content of the cerebrospinal fluid has decreased. The general condition has been remarkably good under this treatment, and no relapses have occurred, even though such relapses are well known in the natural history of heredopathia atactica polyneuritiformis (Try and Stokke[20]).

Does the accumulated phytanic acid cause some or all of the clinical manifestations in this disease? Results of feeding experiments in animals do not indicate a relationship between phytanic acid accumulation and clinical manifestation. There may, however, be important species differences. In autopsied cases of heredopathia atactica polyneuritiformis, phytanic acid has accounted for 8.5 per cent of the total

brain fatty acids and 13.8 per cent of the fatty acids of the sciatic nerve. On the other hand, feeding experiments in polecats and rats resulted in incorporation of only trace amounts of phytanic acid (0.1 per cent of the fatty acids) into the brain lipids.[20] Therefore my personal belief is that the negative results of feeding experiments in animals do not rule out a direct pathogenetic role of phytanic acid in this disease.

The results of dietary treatment in our patients and in some others with heredopathia atactica polyneuritiformis lead us to believe that the phytanic acid accumulation may have a direct pathogenetic effect. In view of the remitting course of the disease and the few patients studied, we fully realize that our observations so far are suggestive only. Definite conclusions as to the relationship between phytanic acid accumulation and clinical manifestations must await further studies.

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TRANSFERRIN, THE CENTRAL FIGURE IN THE METABOLISM OF IRON BY MAN. A PROGRESS REPORT.

ANATOLY BEZKOROVAINY

INTRODUCTION

Most textbooks of hematology visualize the overall fate of iron in the human organism as shown in Fig. 1. It is seen that transferrin occupies the central position in this scheme of events. Transferrin is a protein of serum, synthesized in the liver, which binds two iron atoms in each protein molecule.[1] One bicarbonate ion for each iron atom is also bound by this protein. Transferrin has no prosthetic groups such as heme, and the iron is bound entirely through the participation of amino-acid side-chains. The iron is in the ferric state and the iron-transferrin complex is extremely strong[2] with a binding constant of near 10^{30}. The complex has a characteristic pink color, with an absorption maximum at 460 mμ, and this property of transferrin serves as a convenient method for the testing of its iron-binding ability or capacity. The binding of iron is best at pH 8-9, and below pH 6.5 transferrin loses its iron. This process can be expedited by adding a chelating agent such as citrate to the iron-saturated transferrin at pH values below 7. The iron-free transferrin is colorless.

It was believed for many years that the amount of dietary iron absorbed is directly related to the degree of saturation of transferrin with iron.[3] However, the transferrin is saturated with iron to a much greater extent than normal (normally transferrin is 30 to 50 per cent saturated with iron) in iron storage diseases, yet absorption of iron continues unimpeded in such cases. It has, therefore, been postulated that the amount of iron absorbed from the gut does not depend on the overall degree of saturation of transferrin with iron but on the availability of a certain kind of iron-binding site on the transferrin molecule.[4] In effect this postulates that physiologically the two iron-binding sites of transferrin are not identical.

The transfer of iron from transferrin to immature red cells (reticulocytes) has been investigated in some detail. Free iron is taken up by the cells very sluggishly and, when taken up, cannot be used by cells for the synthesis of hemoglobin. The iron must thus be attached to transferrin if the iron is to be utilized by the reticulocytes. The overall iron transfer process consists of three steps: (1) the attachment of transferrin to the reticulocyte; (2) the transfer of iron from transferrin to the reticulocyte; (3) the release of transferrin from the reticulocyte.

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From the Department of Biochemistry, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Anatoly Bezkorovainy, Ph.D., Associate Biochemist

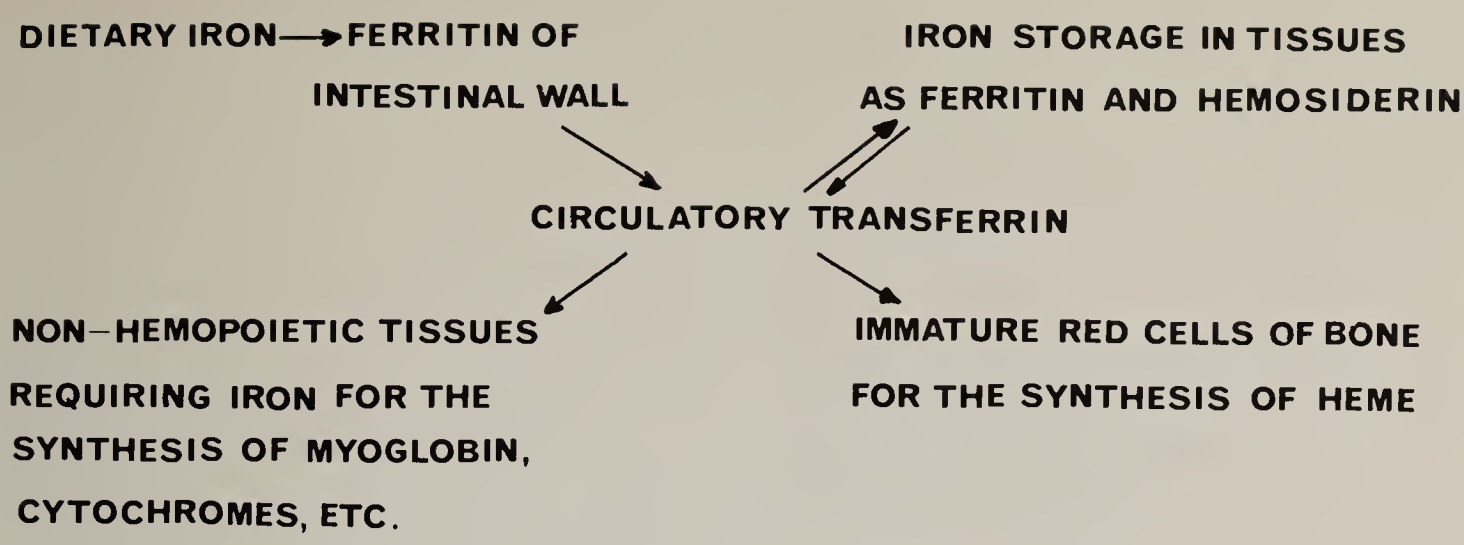
DIETARY IRON
FERRITIN OF INTESTINAL WALL
IRON STORAGE IN TISSUES AS FERRITIN AND HEMOSIDERIN
CIRCULATORY TRANSFERRIN
NON-HEMOPOIETIC TISSUES REQUIRING IRON FOR THE SYNTHESIS OF MYOGLOBIN, CYTOCHROMES, ETC.
IMMATURE RED CELLS OF BONE FOR THE SYNTHESIS OF HEME

Fig. 1—A simplified scheme of iron metabolism in man. Arrows indicate the migration of iron.

In this connection, two interesting phenomena have been noted: iron-free transferrin cannot become attached to the reticulocyte, and the transferrin released from the reticulocyte (step 3) after the transfer of iron to the reticulocyte still contains iron in significant amounts. These facts were interpreted to mean that (a) during the process of iron binding, the transferrin assumes a conformation different from that of the iron-free matrial, and thereby new sites used to bind transferrin to the reticulocyte are created; and (b) that the two iron-binding sites of transferrin are unequal with respect to their ability to donate iron to the reticulocyte. It was moreover postulated that the iron-binding site of transferrin primarily responsible for the uptake of iron from the gut is the same site that is primarily involved in the transfer of iron to the reticulocyte.[5]

Some years ago we decided to focus our attention on the following two problems of biochemical interest: 1. In view of the suggestion that the conformation of the iron-saturated transferrin is different from that of the iron-free transferrin molecule, we sought to define such differences in terms of hydrodynamic constants (sedimentation, diffusion, viscosity), helical contents of the two varieties of transferrin, and the reactivity of their amino acid side-chains; and 2. The structure, in terms of conformational parameters and amino acid sequences, of the two iron-binding sites (active sites) of transferrin.

Isolation

Before much work can be done on the chemistry of a biological substance it must first be isolated in a reasonably homogeneous form. Isolation of some proteins is extremely easy (e.g. albumin and β-lactoglobulin), others require tedious and lengthy procedures.

We commenced our work on transferrin by developing a reasonably rapid two-step procedure for its isolation. Previous work on the isolation of glycoproteins from human and bovine plasma[6] showed that transferrin was precipitated from human or bovine plasma by the method shown in Fig. 2.

The ammonium sulfate precipitate containing transferrin was then chromatographed on DEAE-cellulose by the method of Sober and Peterson[7] or Gordon[8] to yield electrophoretically homogeneous transferrin preparation. Later a gel filtration step (Sephadex G-200) was added to this procedure to separate monomeric transferrin from a small amount of aggregated transferrin

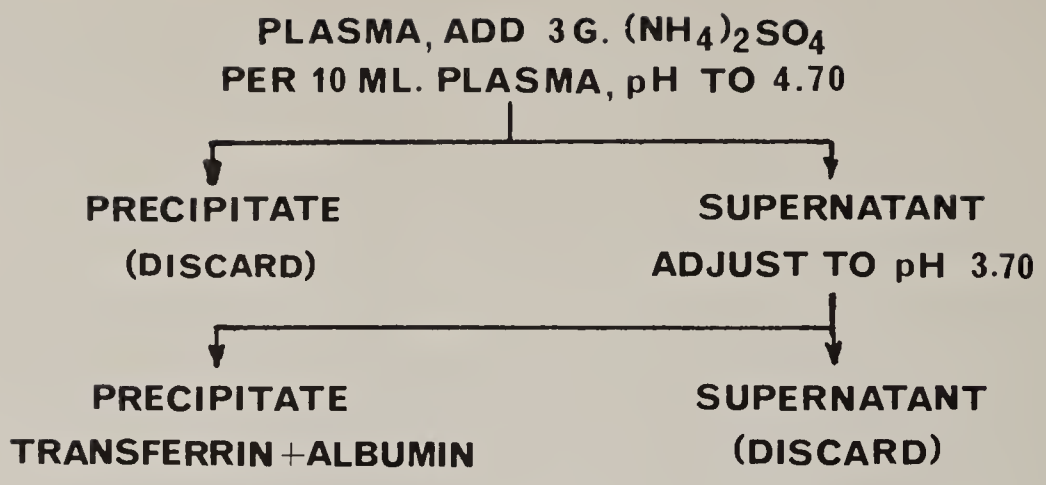

Fig. 2—Fractionation of plasma with ammonium sulfate for the isolation of transferrin.

that was occasionally found in our preparations.[9]

In 1967 transferrin became commercially available through Hoechst Laboratories at a reasonable price. The quality of this preparation proved to be comparable to that of our preparation. Injected into rabbits, the commercial transferrin acted as a single antigen when tested by the immunoelectrophoretic and Ouchterlony methods. In some instances, the commercial product was chromatographed on Sephadex G-200 gels to remove the small amount of transferrin aggregate material. The availability of commercially-prepared transferrin permitted us to devote much more time to the investigation of the properties of transferrin without the need to spend many tedious hours at its isolation.

Physical Properties

Physical properties of transferrin were investigated in our laboratory in an attempt to discover conformational differences between the iron-saturated and iron-free materials. Table I indicates the physical constants obtained for the two proteins at pH 7.[10,11]

Although these values indicate that iron-saturated transferrin has a more disordered shape (higher axial ratio) than does iron-free transferrin, all respective parameters except optical rotation for the two transferrin species are within experimental error of each other, and the differences observed in the axial ratios may be considered not significant. It may thus be stated that any conformational changes that may take place in

TABLE I.
PHYSICAL PARAMETERS OF IRON-FREE AND IRON-SATURATED TRANSFERRINS

	Iron-saturated	Iron-free
Sedimentation constant (S-units)	5.38	5.31
Diffusion constant (cm^2/sec. $\times 10^7$)	5.75	5.54
Intrinsic viscosity (ml/g)	4.0	4.1
Partial specific volume (ml/g)	0.722	0.722
Molecular weight	86,000	87,000
Optical rotation	$-53°$	$-47°$
β-value $\times 10^6$	2.15	2.13
Axial ratio	1 : 3	1 : 2

transferrin upon the binding of iron are too subtle to be detected by the hydrodynamic methods used in our laboratory.

The change observed in optical rotation may indicate a conformational difference; however, it probably reflects also the introduction of additional asymmetric centers due to the complexed iron and the restricted rotation of amino-acid side-chains involved in the binding of iron.

There exists even at the present time, disagreement regarding the true molecular weight of transferrin. Earlier investigations[1,12] found the molecular weight to be near 90,000. Originally we found a value of 93,000.[9,10] With the introduction of Sephadex G-200, values of 86,000 were obtained in our laboratory.[11] Other workers got values of 67,000[13] and 74,000.[14] More recently, values of 83,000[15] and 80,000[16] have been reported. The determination of molecular weights of transferrin in dissociating solvents or following succinylation[18] gave values of 82,000 and 79,000 respectively in our laboratory. We feel that these discrepancies are due to the ability of native transferrin to form dimers or higher oligomers in dilute buffer solutions and that the most meaningful molecular weight values should probably be obtained either in the dissociating solvents or following succinylation of this protein. The true molecular weight of transferrin is, in our opinion, near 80,000.

Two other sets of experiments may be mentioned in connection with the differences in conformation between free and iron-saturated transferrin. It was found in our laboratory[18] that succinylation of free transferrin resulted in the modification of 63 amino groups per molecule of protein. The same reaction using iron-saturated transferrin modified only 53 groups. Thus, the attachment of iron to transferrin resulted in the "masking" of 10 amino groups per molecule of transferrin. Further work revealed that the 10 amino groups are already masked by the binding of a single iron atom per protein molecule, and that the binding of the second iron atom does not result in additional amino groups becoming "buried."[19] At the same time, Ulmer[20] found that tritium exchanged with hydrogen much more readily in the free transferrin than in iron-saturated transferrin. Both sets of experiments may thus indicate that transferrin assumes a more compact shape upon the binding of iron, and that this transition occurs upon the binding of a single iron atom per protein molecule.

Quaternary Structure of Transferrin

Since the physiological experiments mentioned above have suggested that the active sites of transferrin were different, it was logical to attempt to separate the transferrin molecule into two halves, each having one iron-binding site. There were several suggestions in the literature that transferrin might consist of two polypeptide chains joined by disulfide bonds. These opinions arose from the fact that transferrin had two iron-binding sites and two carbohydrate chains.[21] In fact, one investigator claimed to have dissociated transferrin into two identical subunits by either 8M urea treatment or by reduction-alkylation.

We at first attempted to dissociate transferrin into subunits with 8M urea or 6M guanidine-HCl.[22] These reagents bring about the dissociation of all noncovalent interactions in proteins. The results are shown in Table II.

The data in Table II suggest that no dissociation of transferrin into subunits takes place in 8M urea or 6M guanidine-HCl, and that transferrin simply becomes denatured under these conditions.

In this connection it was interesting to examine the relative stabilities of free and iron-saturated transferrin to dissociating solvents.[22] Table III shows the susceptibility of the two transferrins to denaturation.

It may be seen from Table III that iron-saturated transferrin is much more stable than iron-free transferrin with respect to denaturation. This may again indicate that the iron-saturated trans-

TABLE II
PHYSICAL PROPERTIES IN DISSOCIATING SOLVENTS

	8 M Urea	6 M Guanidine-HCl
Sedimentation constant (S-units)	4.8	4.8
Diffusion constant ($cm^2/sec. \times 10^7$)	4.6	4.5
Intrinsic viscosity (ml/g)	20.2	14.7
Molecular weight	93,000	89,000
Optical rotation	−100°	−100°
β-value $\times 10^6$	—	2.59

ferrin has a more compact shape than iron-free transferrin.

The next step in the elucidation of the quaternary structure of transferrin was its reduction and alkylation, a process designed to break all disulfide linkages. We used the procedure of reducing with mercaptoethanol and alkylating the -SH groups produced with iodoacetic acid. The molecular weights were then determined in the presence of 4-8M urea[17] or following complete succinylation.[18] The results are given in Table IV. The data in this table indicate the transferrin was not dissociated into smaller fragments by the rupture of its disulfide bonds, and that, instead, it had assumed a random coil conformation.

TABLE III
SUSCEPTIBILITY TO DENATURATION OF FREE AND IRON-SATURATED TRANSFERRINS AFTER STANDING IN THE DISSOCIATING SOLVENT FOR 24 HOURS

Optical rotation	Free Transferrin	Iron-saturated Transferrin
Native	−49°	−53°
2 M urea	−49°	−52°
4 M urea	−84°	−54°
6 M urea	−99°	−55°
8 M urea	−100°	−88°
Absorbance at 465 mμ		
Native	colorless	0.605
2 M urea	colorless	0.596
4 M urea	colorless	0.597
6 M urea	colorless	0.585
8 M urea	colorless	0.061

Chemical Nature of the Active Sites

The availability of chemical reagents specific for the modification of specific amino acid side-chains in proteins prompted us to investigate the nature of the amino acid side-chains involved in the binding of iron. Since the separation of transferrin into half-molecules, each having one iron-binding site, had not yet been accomplished, it was necessary to use the intact transferrin molecule for these studies and to make an assumption, as a working hypothesis, that the chemical structure of the two binding sites is identical.

Tyrosyl Groups

The role of tyrosyl groups in the binding of iron was investigated via nitration with tetranitromethane. It was found[23] that the nitration of free transferrin abolished its iron-binding properties without causing any changes in its tertiary structure. Nitration of iron-saturated transferrin, on the other hand, resulted in the modification of six fewer tyrosyl residues than in the iron-free material without the loss of iron. The six residues were then nitrated after the removal of iron. We concluded that three tyrosyl residues were required to bind each iron atom to transferrin. A study was also made on the iron-binding properties of partially nitrated iron-free transferrin. The nitration of eight tyrosyl residues resulted in the gradual loss of

TABLE IV
PROPERTIES OF REDUCED-ALKYLATED TRANSFERRIN

	In 4 M urea	Succinylated
Sedimentation constant (S-units)	2.87	2.58
Diffusion constant (cm^2/sec. $\times 10^7$)	3.18	2.69
Intrinsic viscosity (ml/g)	48.4	57.5
Molecular weight	80,000	84,000
β-value $\times 10^6$	2.60	2.48

50% of the iron-binding activity of transferrin. Nitration of additional four residues was not followed by any further loss of iron-binding acitivity. However, iron-binding activity was further decreased as the remainder (12-20) of the tyrosyl groups were nitrated. The existence of such a plateau region (8-12 tyrosyl residues nitrated) indicated that the two active sites were not identical with respect to the reactivity of their tyrosyl residues.

Histidyl Residues

Imidazole groups were modified by alkylation with bromoacetic acid at pH 6.5. No loss of iron-binding activity was seen upon the alkylation of 10 histidyl groups of apo-transferrin. Beyond that, iron-binding activity was rapidly lost until, with 14 histidyl groups modified, no iron-binding activity remained.[23] Recent, as yet unpublished, data showed that alkylation of iron-saturated transferrin results in the loss of iron from transferrin. We concluded from the above experiments that two histidyl residues are involved in the binding of each iron atom in transferrin.

Amino Groups

The role of amino groups in the binding of iron by transferrin is being investigated by R. Zschocke and, in part, by M. Chiao in this laboratory. It was found that unfortunately the most specific reagent for the modification of amino groups, trinitrobenzene sulfonic acid, causes an aggregation of transferrin when the majority of amino groups are modified. Reductive alkylation of transferrin with formaldehyde and sodium borohydride has been able to modify, so far, only 80% of all the amino groups of transferrin, and the transferrin has, under these conditions, retained its full iron-binding activity.

Succinylation of apo-transferrin resulted in a loss of its iron-binding activity.[18] This was, however, attributed to a change in conformation of transferrin brought about by the succinylation, rather than specific modification of the active sites. Succinylation of iron-saturated transferrin did not result in a loss of its iron, nor was the conformation of the protein significantly changed. On the other hand, it will be recalled (see above) that only 53 of the available 63-65 amino groups were succinylated in the iron-saturated transferrin. The iron could be removed from and reconstituted with this transferrin preparation. However, if the remaining ten amino groups were trinitro-phenylated, the transferrin lost its iron-binding activity without significant changes in its conformation. We conclude from these studies that the more reactive amino groups of transferrin do not take part in the binding of iron by transferrin. However, the far less reactive amino residues appear to be involved in the binding of iron, perhaps indirectly, by stabilizing the iron-transferrin complex.

The ability of the iron to protect the active site of transferrin against succinylation was used to advantage by R. Zschocke in preparing an antiserum to the region of transferrin molecule containing the active sites. Thus, iron was removed from the succinylated iron-saturated protein (53 amino groups modified) which then was injected into rabbits. The antiserum obtained was treated by the fully succinylated apo-transferrin (63-65 amino groups modified) to absorb antibodies to the succinyl residues. The resulting antiserum did not react with succinylated transferrin, but gave precipitin lines in the Ouchterlony system with the partially succinylated transferrin (53 amino groups modified) and native transferrin. A partial-identity, precipitin-line pattern was seen with the two antigens.[24]

Carboxyl Groups

Carboxyl groups of transferrin were modified by coupling with glycine methyl ester or with glucosamine via the carbodiimide reaction. The introduction of 10 glycine or 4 glucosamine residues into a protein molecule completely abolished its iron-binding activity without any effect on its tertiary structure.

The introduction of additional glycine or glucosamine residues into the protein resulted in its insolubilization at neutral and slightly alkaline pH-values.[25] It is clear that the carboxyl groups of transferrin play an important role in its stabilization under physiological conditions, and also in stabilizing its iron-binding sites. The carboxyl groups having the latter function are apparently very reactive.

The iron-binding site of transferrin may be visualized to consist of two tyrosyl residues, two histidyl residues, and a bicarbonate ion. Amino and carboxyl groups are also involved in some capacity in the binding of iron by transferrin. Their role may be an indirect one, such as maintaining the proper conformational pattern of those sections of the polypeptide chain that contain the active site of transferrin.

Prospects for the Future

It is clear from the foregoing discussion that the goals set at the beginning of our investigations have not yet been achieved, although considerable progress has been made.

It is still uncertain what conformational differences, if any, exist between the iron-free and iron-saturated transferrins. Obviously, more sophisticated methods are needed to study this, and we hope in the near future to gain access to optical rotatory dispersion and dichroism methods to study the α-helix-β-helix-random coil relationships in the two types of transferrins.

Elucidation of the chemical structure of the active sites for transferrin is also yet to be achieved. We know something of the nature of amino acid side-chains involved in the binding of iron by transferrin. However, nothing is yet known about the differences in the chemical structures of the two binding sites or their environments (*e.g.*, amino acid sequences). The availability of the antiserum to the active site region of transferrin will, hopefully, be of help. We will attempt to fragment the transferrin molecule into smaller peptides enzymatically or chemically and identify those peptides that bind to the antiserum, as representing the active sites. The amino acid sequences of the isolated peptides, and hence the difference between the two types of active sites, may be established.

Clinical Significance

A knowledge of the chemistry of transferrin should be useful in the treatment of

several disorders of iron metabolism in man. For example, in patients with the iron-storage disease, hemochromatosis, the infusion of transferrin results in the mobilization of iron from its storage sites in liver. However, very little of the mobilized iron can be discarded, since the organism is geared primarily for the conservation of iron rather than its excretion. The knowledge of the structure of the active sites of the transferrin molecule will permit a laboratory synthesis of small compounds that would specifically chelate iron. Injection of such a compound (dummy transferrin) into the hemochromatotic patient would mobilize the iron from its storage sites. Due to the small size of such a complex, the kidneys would, in all probability, be able to clear it from the organism.

On the other hand, if we had the knowledge of the chemical structure of the sites of transferrin that are responsible for its attachment to immature red cells, we could synthesize analogues of such sites. The analogues, when injected into an individual, might compete with transferrin for a place on the surface of the immature red cells, thus retarding the transfer of iron from transferrin to the immature red cell. Such drugs may perhaps be used for the symptomatic treatment of polycythemia vera.

SUMMARY

Several aspects of the structure of transferrin have been investigated. Hydrodynamic methods were not able to demonstrate any appreciable difference between iron-free and iron-saturated transferrin; however, chemical evidence suggests that the latter form is more compact than the former. Transferrin apparently exists as a single polypeptide chain, since it cannot be dissociated into smaller polypeptide chains either by reagents that break physical bonds only, or by breaking the disulfide bonds. The modification of tyrosyl, histidyl, amino, and carboxyl groups abolishes the iron-binding activity of transferrin. Tyrosyl and histidyl groups are apparently directly involved in the binding of iron, whereas amino and carboxyl groups are believed to play a role in stabilizing the iron-transferrin complex. There is some promise for application of these concepts in the clinical management of such problems as hemachromatosis and polycythemia vera.

ACKNOWLEDGEMENTS

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THE PEDIATRIC ASSISTANT: CREATION OF A NEW ROLE FOR TODAY'S CHILD HEALTH CARE CRISIS

Albert L. Pisani

INTRODUCTION

A severe manpower shortage in the face of ever-increasing demands for health care has been recognized for some time.[1–3] In 1966, there were 399 practicing pediatricians in Chicago,[4] 514 practicing pediatricians in the State of Illinois,[5] and only 10,089 practicing pediatricians in the whole United States.[6] In addition, whereas in 1930, 85% of the United States' physicians were in general practice, this group today comprises only 35%, and it is estimated that of this year's medical graduates, only 14 to 18% will go into general practice.

The vast majority of child health services are provided by either general practitioners or by pediatricians. In 1940, there were 337 practicing pediatricians and general practitioners for each 100,000 children under the age of fifteen. By 1963, this ratio had fallen to 133 per 100,000.[7]

With the shortage of child care physicians now estimated at approximately 70,000,[8] it is being generally appreciated in this country that there IS a crisis in child care, and this is a matter of critical concern to all.

The setting of future goals to meet the situation should be a realistic process rather than merely an exercise in rhetoric. It should be quite obvious that if goals are to be reached, the resources for their implementation must be either available or potential.

From the Section of Ambulatory Pediatric Services, Division of Pediatrics, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Albert L. Pisani, M.D., Associate Attending Pediatrician; and Director, Section of Ambulatory Pediatric Services

Presented at the Thirty-ninth Annual Meeting of the Tri-State Hospital Assembly, Chicago, Illinois, April, 1969

There appear to be three methods by which child medical care may be increased: (1) Increase the number of physicians rendering child care, (2) Increase the productivity and efficiency of physicians caring for children, and (3) Increase the utilization and efficiency of available paramedical personnel.

Past experiences indicate that there is a time span of at least ten years from the planning stage of a new medical school to the graduation of its first class. Three more years must be added to complete the required training program in pediatrics. It is the opinion of many in the field, including pediatrician William Stewart, Surgeon General of the United States, that the physician-manpower needs cannot be met.[9]

Increasing the productivity and efficiency of physicians caring for children can be accomplished through careful analysis and trial to identify those functions which *must* be performed by a pediatrician and those which can be per-

formed satisfactorily by a less completely trained person. The pediatrician, with over a decade of education beyond the high school level, should use his time in the performance of those tasks which he alone is prepared to do. He is otherwise practicing in an inefficient manner. Lack of stimulation may cause him to lose his highest professional skills and the incentive to continue his own education may languish. As Fein[12] has pointed out, a mere 5% increase in the productivity of existing private general practitioners and pediatricians would add the equivalent of 3,900 physicians—more than the annual number now entering general practice and pediatrics.

Routine child care has changed greatly in the last quarter century. With the disappearance of many infectious and nutritional disorders, current emphasis is on preventive pediatrics. Various studies have revealed that well-child examinations, or preventive pediatrics, occupy 30% to 65% of a pediatrician's time. This has been true for over a quarter of a century. Studies by C. A. Aldrich[10] in 1934 and Bergmen[11] in 1966 indicated strikingly similar experiences. Two-thirds of this *preventive pediatrics* could be provided by persons with less intensive training, thereby increasing the productivity of existing pediatricians by 33%. This would add the equivalent of 3,000 practicing pediatricians to the nation's manpower pool.

More efficient utilization of paramedical personnel in this field must be seriously considered. One of the recommendations of the National Advisory Commission on Health Manpower was that nursing be made a more attractive profession through appropriate utilization of nursing skills and increased levels of professional responsibility. Unyielding adherence to traditional attitudes on the part of physicians as well as nurses has prevented nurses from developing their full potential.

During the past two years, the Mile Square Health Center in Chicago has utilized nurses as pediatric assistants. Nurses who have functioned in this role have found it very satisfying. The opportunity to provide primary, rather than merely auxiliary, patient care has permitted them to utilize their training more fully and provided them with more meaningful patient care experiences.

Pediatric assistants are capable of performing the following functions:

1. Routine History-Taking
2. Routine Physical Examination of Infants
3. Anticipatory Guidance
   a. Accident Prevention
   b. Growth and Development
4. Infant Nutrition
5. Immunization Procedures
6. Treatment of Minor Disorders and Diseases (within guidelines prescribed by supervising pediatricians)
7. Exit-interviews with mothers. (Pediatric assistants can interpret physicians' orders, handle referrals, and use this opportunity for teaching. They can discuss the presenting problems and give guidance and advice. Pediatric assistants are equipped to deal with a great many problems by virtue of their developed skills and experiences.)

Experience with this program has led us to believe that we may go one step further in utilizing available manpower to maximum efficiency. It is our feeling that even nurses are overtrained for this particular task.

We are looking forward to the development of a training program for non-nurse pediatric assistants, under the co-sponsorship of Presbyterian-St. Luke's Hospital and an appropriate educational

facility. The length of such a program will be three calendar years, leading to an Associate in Arts degree. The curriculum will consist of formal classroom lectures, seminars, conferences, and supervised clinical experience. The majority of classroom lectures will be held at the educational facility, and clinical experience will be under the direction of the Hospital. This clinical experience will include participation in the newborn nurseries, general pediatric outpatient clinics, pediatric specialty clinics, adolescent clinics, and emergency care. Students will also serve preceptorships in the offices of practitioners.

Emphasis throughout the clinical training program will be on *practical* ambulatory pediatric care, which now accounts for 95% of pediatric practice, rather than on learning factual and conceptual information which is relevant primarily for those expecting to deal with the rare, the exotic, the more severe, and the more advanced disease states.

Upon completion of the prescribed program, the pediatric assistants should possess sufficient knowledge, skills and experience to conduct a good medical interview, complete a routine physical examination, arrive at a differential diagnosis, form an impression, and recommend appropriate further studies and, if necessary, refer patients to a pediatrician for consultation or further management. The pediatric assistants should be capable of performing routine diagnostic tests and procedures and offer counsel in routine problems. They will be given far greater experience in the area of preventive pediatrics and crisis-care than is received by the medical student during his pediatric experience which usually consists of a total of six to twelve weeks.

As added "quality control" pediatric assistants, at all times during training as well as after graduation, will function under the direction and supervision of a physician. The presence of pediatric assistants in the office of private practitioners should result in improved patient care and more efficient and more effective utilization of the time and professional skills of the practicing pediatrician.

The pediatric assistant should be capable of handling satisfactorily almost all of the telephone calls previously directed to the pediatrician. This alone should lead to a decrease in the morbidity and an increase in the longevity of the practicing pediatrician!

To the physician who prefers solo practice over associate practice, the pediatric assistant offers the advantage of permitting him to preserve his independence and control over his practice. Although the pediatric assistant is a professional colleague, the independence and status of the physician is maintained.

Graduates of this program should possess sufficient skills and experience to prevent, diagnose, and treat the vast majority of common medical problems in childhood. It is estimated that upon completion of the prescribed course of study, the pediatric assistant will possess the knowledge and skill necessary to care for 75-80% of patients seen in a typical pediatric practice.

PLACEMENT

Graduates should be qualified to function in the office of private practitioners or health department clinics under the supervison of a physician. Medical care outside the physician's office or clinic can be rendered only under the specific directions of the physician in each individual situation. In-patient hospital privileges of the pediatric assistant should be limited to care of the normal newborn infant.

Before this system can be widely used, certain obstacles must be faced and overcome. One question arises: Since well-baby care is more concerned with

guidance of the mother than care of the baby, would a mother receive the same reassurance from a non-physician? Public education may be required in this regard.

It will be necessary to update restrictions of the medical practice act. Maximum utilization of personnel is hindered by outmoded limitations which have been incorporated into licensure certification codes for people in health professions. Although originally designed to insure a high quality of patient services, these currently work with telling effect to defeat efficient utilization of available health manpower resources.

CONCLUSION

It is sociologically and economically unsound to utilize persons at a lower level than that for which their training and experience qualify them, nor should persons be given tasks at a higher level than that for which they are capable. It is imperative, moreover, that this nation use its existing supply of health manpower to obtain maximal benefits. The educational program now being contemplated at Presbyterian-St. Luke's Hospital promises to be a significant contribution to the solution of these problems.

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BOOK REVIEW

RADIOLOGY OF BONE DISEASES by George B. Greenfield. Cloth, 465 pp., with 825 illustrations and numerous tables. Philadelphia and Toronto: J. B. Lippincott Company, 1969. $34.00.*

George B. Greenfield's recent book, *Radiology of Bone Diseases*, presents a new approach to the subject. Instead of grouping diseases according to etiology, he analyzes them according to their predominant roentgenologic features such as loss of bone density, alteration of bone texture, changes in size and shape of bones, *etc.* In addition to the 825 excellent illustrations, there are numerous exhaustive tables which greatly aid in the differential diagnosis. The clinical and laboratory findings are presented in crisp informative text which also reviews the literature and unearths many rare entities.

The book has few errors and few upside-down illustrations for a first edition. In general it is an excellent contribution, and I predict it will see many editions. JOHN W. CLARK

**EDITOR'S NOTE: George B. Greenfield, formerly resident in Radiology at Presbyterian-St. Luke's Hospital, is now Chairman of the Department of Radiology at Mount Sinai Hospital, Chicago, Illinois, and Chairman of the Department of Radiology at the Chicago Medical School, Chicago, Illinois.*

ABSTRACTS

OF PUBLICATIONS BY THE STAFF

Farago, Peter J., Seigel, Elizabeth W., and Robinson, John E.: Hospital-college Program Taps New Manpower Source. *Hospitals, 43: 73-77, Sept. 1969.*

The critical shortage of allied health manpower in this country will become even more serious as the population expands. Although the extent of this need in the Chicago area still is undetermined, there are excellent statewide parameters in the report "Education in the Health Fields for the State of Illinois Board of Higher Education." The Chicago City College and its affiliated clinical facilities are taking the first step toward filling this need. These facilities include Presbyterian-St. Luke's Hospital, West Side Veteran's Administration Hospital, Jewish Home for the Aged, St. Mary of Nazareth Hospital, University of Chicago Hospital, Edgewater Hospital, Wesley Memorial Hospital, and the University of Illinois Research and Educational Hospitals.

To date, emphasis has been mainly on short-term programs, but more upper-level programs will be offered in the future to provide career ladders and to improve lateral and vertical job mobility.

Problems yet to be solved include: broadening the recruitment base, reducing the dropout rate, and providing for more articulation with college-level programs.

The real results can be evaluated only after the program has been in operation for a longer period of time. The success of the program to date, however, has been due to excellent communication and cooperation between the clinical institution and the junior college.

Lepper, Mark H.: Scientific Issues and Research Needs Concerning Microbial Ecology and the Use of Drugs in Feeds: The Use of Drugs in Animal Feeds. *Nat'l Acad. Sci. Publ., No. 1679, 1969.*

The nature of chronic diseases and their expected rates in humans resulting from a procedure, such as the use of drugs in animal feeds, make it difficult to know the safety of such procedures. The lack of evidence of definite adverse effects can easily be explained by the fact that they have not been looked for with sensitive methodology. This should include (a) grade sequential introduction of new procedures so that population carefully selected as to size are observable; (b) adequate surveillance of all types of disease. With computer technology the latter should become increasingly feasible.

That changes in microbial ecology can accompany the use of chemotherapeutic agents in a proportion of the population has been repeatedly demonstrated in a variety of situations and with a variety of drugs. These simple observations lead to a series of questions, none of which can be answered with great precision; hence they constitute the scientific issues and the needs for research and surveillance. Two overview questions focused upon are: (a) are the changes observed causally related to the use of the drug? (b) are the changes of serious import?

Limitations of laboratory observations have precluded the opportunity for direct observations of causal relationship. Much indirect evidence has suggested that the increases in resistant organisms are causally related to the level of drug use. Such changes are of potential importance.

Suhs, Richard H., Lumeng, James L., and Lepper, Mark H.: An Experimental Immunologic Approach to the Induction and Perpetuation of Chronic Bronchitis. *Arch. Environ. Health. 18: 564-573, April, 1969.*

An experimental model is presented utilizing an immunological pathogensis involving rabbit tracheo-bronchial mucosa as antigen in producing histologic evidence of chronic bronchitis in rabbits as compared to control groups. The implications of this model are also discussed.

Cutler, Sidney J., Asire, Ardyce J., and Taylor, Samuel G., III: Classification Of Patients With Disseminated Cancer of The Breast. *Cancer 24: 861-869, Nov. 1969.*

Some of the presenting findings of patients with breast cancer at the time of the initial diagnosis of disseminated disease have been collected on 920 patients from eight centers. These findings have been correlated with survival. This study indicates that the interval between initial diagnosis of the primary disease and the clinical diagnosis of disseminated cancer, and the number and type of organ sites involved, influence the survival from the onset of metastases. It also indicates that if more complete information on the history and clinical course of breast cancer patients were available in studies involving therapeutic trials than presently is used in cooperative studies, the results of such studies might be more meaningful. A useful staging of disseminated cancer for the evaluation of therapeutic trials may be developed for several types of disseminated cancer by utilizing such information.

Christian, Joseph R., Pisani, Albert L., and Shannon, Iris: Crisis in Child Care. *Illinois Med. J., 135: 589, May 1969.*

In 1966 there were 399 practicing pediatricians in Chicago, 514 practicing pediatricians in the state of Illinois, and only 10,089 practicing pediatricians in the United States.

In 1940 there were a total of 337 practicing pediatricians and general practitioners per 100,000 children under the age of 15. By 1963, this ratio fell to 133 per 100,000.

At least 50% of a pediatrician's time is spent in well-baby care or preventive pediatrics, two-thirds of which could be provided by a person with less extensive training.

The Mile Square Health Center, in Chicago, since its inception, has utilized nurse-associates to provide primary pediatric care. Functions which the nurse-associates routinely perform include: routine history taking, routine physical examination in infants, anticipatory guidance in accident prevention and growth and development, infant nutrition, immunization procedures, pediatric screening, exit interviews with mothers, and treatment of minor disorders and diseases.

The ultimate goal in manpower utilization should be to have each worker on the health team performing consistently at his or her highest level.

Hyde, John S.: Symposium on Pediatric Allergy (*Foreword*). *Ped. Clin. N. Amer., 16: 1-7, Feb. 1969.*

Pediatric allergy has progressed during the past 22 years to a point where the originators of the specialty are familiar names in pediatrics and allergy. In order to update information in the field, the editor has handpicked pediatricians, mostly second generation successors, who provide both theoretical and practical approaches to the discipline. Controversy about what is immunologic and what is not immunologic permeates the seminar.

Many contributions have been made during the past five years to the understanding and care of both acute and chronic allergic disorders by allergists interested in pharmacology, physiology, psychology, pathology, and microbiology. Meanwhile immunologists have helped clarify the role of immunoglobulins *A* and *E* in immediate hypersensitivity and the cellular phenomena of delayed hypersensitivity. Dysgammaglobulinemia in childhood is associated with a high incidence of allergic disease of the respiratory tract. By contrast, agammaglobulinemia is more often associated with eczema and disorders such as rheumatoid arthritis, systemic lupus erythematosus, regional ileitis, and thrombocytopenia.

It is possible that the capacity of the individual's leukocytes to react to anti-IgE antibody and release histamine may constitute the long-sought genetic marker of atopic sensitivity. On the other hand there may be a basic enzymatic defect involving adenyl cyclase in asthmatics and probably other atopic persons. This results in unusual susceptibility to a number of pharmacophysiologic agents which are concerned primarily with homeostasis, inflammatory defense, and releasing such agents. Histamine, SRS-A, and the kinins arise from several cell sources and are activated by a number of different mechanisms, resulting in an allergic symptom complex.

The authors are concerned with the practical approaches and management of disabling allergic disorders most frequently encountered. These patients require careful investigation and follow-up. Thus, on Monday the challenge for the pediatric allergist may be bloody stools in a milk-sensitive infant; on Tuesday the role of complement in Henoch-Schoenlein purpura is discussed; on Wednesday a patient who had life-threatening yellow jacket reaction is referred. And as the week passes, one is called in consultation for the child with recurrent pneumonitis and wheezing; a teenager who is hospitalized for the third time in a year, after a near-fatal reaction to aspirin; then for a chronic dermatologic disorder, this time dermatitis herpetiformis; and by Sunday for a cyanotic semicomatose eight-year-old with status asthmaticus complicated by atelectasis and pneumothorax.

Rapid changes in the field of allergy have been documented in the Pediatric Clinics of North America about every five years. In this edition many of these changes have been put into perspective and it is the first issue to have been completely authored by pediatric allergists. The wide horizons of the field make it increasingly difficult for the sub-specialist to become preoccupied with one organ system or a limited age group.

Holinger, Paul H. and Schild, Joyce A.: The Zenker's (Hypopharyngeal) Diverticulum. *Ann. Otol., 78: 469, Aug. 1969.*

A Zenker's diverticulum is a well-known and well-documented clinical entity. Many forms of therapy have been described; one type of surgical treatment, the endoscopic diverticulotomy, with an evaluation of over 100 cases reported by Dohlman, and the report of a smaller series by Holinger and Johnston in 1962, has invariably led to controversy. A re-evaluation of this and other procedures in our total experience of 217 cases is presented herein.

Curtin, John W. and Bader, Karl F.: Improved Techniques for the Successful Silicone Reconstruction of the External Ear. *Plast. Reconstr. Surg., 44: 372-377, Oct. 1969.*

A soft silicone framework was used in a series of 42 patients for complete or subtotal external ear reconstructions. The authors' original technique is described, with specific recommendations of a postauricular incision, creation of a dermal "pocket" without overlying suture lines, and use of a mesh-bonded Silastic implant sutured into the desired position. Cosmetic results, on the whole, have been good. There have been only 5 failures in this series.

Curtin, John W., Bader, Karl F., and Ellenby, J.: Reconstruction of the External Ear. Improved Technique Utilizing Silicone Frame Implant. *Plast. Reconstr. Surg., 44: 372-377, 1969.*

Construction of the external ear has been a frustrating experience and generally a bitter latent disappointment to most plastic surgeons and their patients. Children with unilateral or bilateral microtia have been subjected to multiple operations, only to emerge years later with a misshapen unidentifiable structure alongside of the head, vaguely representing an auricle. The chief problem has been to obtain a suitable substitute for the fibrocartilaginous framework of the normal ear. All previous materials (autogenous and hemologous cartilage and bone, bovine cartilage, meniscus of the knee, tantalum and nylon mesh, polyethylene and acrylic) have not been uniformly successful.

In 1965, Dr. Thomas D. Cronin originally described a method of external ear reconstruction utilizing a silicone (Silastic rubber #370) frame implant and recounted his series of eight patients. Personal communications with other surgeons have indicated an unsatisfactory rate of complications, including direct exposure, infection, poor cosmetic result secondary to fluid accumulations, and residual edema of overlying skin.

The authors present a successful series of forty (40) silicone ear implants primarily on difficult microtia cases during the past 24 months. Included are two cases of severely burned ears. The overall results have been most gratifying. Two inserts have been unsuccessful in long-term analysis—each due to external trauma.

The authors feel that this largest collected series of silastic reconstructed ears is due to improved operative techniques detailed in the paper.

Rishpon-Meyerstein, N., Kilbridge, T., Simone, J., and Fried, W.: The Effect of Testosterone on Erythropoietin Levels in Anemic Patients. *Blood., 31: 453-460, Apr. 1968.*

Studies in five patients with various types of anemias have shown that testosterone can increase plasma erythropoietin levels in humans. We suggest that testosterone stimulates erythropoiesis in some anemic patients by this mechanism.

Fried, Walter, Ward, Harry P., and Hopeman, Alan R.: Leiomyoma and Erythrocytosis: A Tumor Producing a Factor Which Increases Erythropoietin Production. Report of Case. *Blood., 31: 813-816, 1968.*

A patient with a leiomyoma of the esophagus and erythrocytosis is reported. Preliminary studies in mice suggest that the tumor contained a substance which was capable of increasing erythropoietin production by the kidneys. Because of the limited amount of tumor available, we were unable to further characterize this substance. We hope that this report will encourage other investigators to repeat and extend these studies.

Fried, W., Knospe, W. H., and Trobaugh, F. E., Jr.: Factors Influencing Proliferation and Migration of Hematopoietic Stem Cells. *J. Lab. Clin. Med., 74: 874, 1969.*

Hematopoietic Stem Cells (HSC) are estimated by the formation of macrosopic spleen colonies after transplantation into lethally irradiated hosts (exocolonization) or after X-irradiation with a leg shielded (endocolonization). Three hundred roentgens of X-irradiation with a leg shielded (300 RLS) was recently shown to increase the endo- but not the exocolonizing potential of HSC in that leg. Since endocolonization is influenced by migra-

tion and proliferation as well as the number of HSC, these functions were studied following 300 RLS. Some mice received 300 RLS, and others were controls. Seven days later, all received 800 R with the leg shielded and 200 R to that leg. The groups were killed 1, 2, 5, 7, 9 and 12 days later, and HSC from spleens, blood, and both legs were assayed exogenously. Two-to-three times more HSC were always found in organs from mice which received 300 RLS than were found in controls. The effect of 300 RLS on the rate of HSC proliferation in both legs was next studied by measuring their sensitivity to vinblastine. Only 45 per cent of exogenously measured HSC remained 14 hours after control mice received vinblastine and approximately 15 per cent after 24 hours. The sensitivity of HSC in the shielded leg to vinblastine was not significantly different from controls 4 and 7 days after 300 RLS. When vinblastine was given 4 days after 300 RLS, only 20 per cent of HSC in the unshielded leg survived 14 hours, and 5 per cent survived for 24 hours. This suggests that proliferation of HSC in nonshielded but not in shielded legs increases after 300 RLS.

We suggest that 7 days after 300 RLS, either depletion of an inhibitor or release of a stimulator of HSC proliferation occurs. HSC in sites with normal or supranormal cell density cannot respond until they emigrate to sites of low cell density.

Vyas, G. N., Sasetti, R. J., Petz, L. D., and Fudenberg, H. H.: Experimental Study of Aldomet-Induced Positive Antiglobulin Reaction. *Brit. J. Haemat. 16: 137-143, 1969.*

Methyldopa polymer binds to red cells. Autologous red cells coated with polymer and polymer mixed with complete Freund's adjuvant induced heterospecific immune responses in rabbits resulting in the production of antibodies against human γ-globulin and other plasma proteins. Therefore methyldopa polymer possibly is a heterospecific hapten. The γG bound to red cells of patients on methyldopa therapy differs from native γG and shows immunologic cross-reactivity with rabbit γG. Two possible mechanisms for producing positive anti-globulin reaction in a large number of patients (10-20 per cent) on methyldopa and infrequent haemolytic anaemia in such patients are discussed.

Gregory, S. A., Fried, W., Knospe, W. H., and Trobaugh, F. E., Jr.: Further Studies on the Effect of Cyclophosphamide on Hematopoietic Stem Cells. Abstract. *Clin. Res. 42: 532, 1969.*

Hematopoietic stem cells (HSC) are measured by their ability to colonize the spleen of lethally irradiated hosts (exocolonization); or to colonize the spleen of the same animal after lethal X-irradiation with a leg shielded (endocolonization). Cyclophosphamide (CY) had been shown to decrease both the endo- and exocolonizing ability of HSC. Whereas the latter recovers after 15 days, the endocolonizing potential exceeds normal after 4. An explanation for this is that CY improves the environment for HSC proliferation. To test this, we compared the rate of regeneration of HSC injected into lethally irradiated CF_1 and CAF_1 mice pre-treated with CY or saline. They received 5 mg. CY or saline intraperitoneally either two hours, one, four or seven days prior to 800r and injection of 2×10^5 normal marrow cells. Nine days later, they were sacrificed and the cells in ½ a femur were injected IV into lethally irradiated recipients. After nine days, macroscopic spleen colonies were counted. In CF_1 mice there was about a fivefold increase in the number of HSC in the

marrow of animals treated with CY two hours, one and four days prior to irradiation as compared to controls. Results in CAF_1 mice were similar. These three hypotheses might explain the results: CY (1) destroys HSC and thereby decreases the titer of an inhibitor to their proliferation, permitting the more rapid growth of transplanted HSC; (2) results in the secretion of a substance which accelerates the rate of proliferation of HSC; (3) alters the radiosensitivity of HSC thereby increasing their number surviving irradiation.

Fried, Walter and Kilbridge, Thomas: Effect of Testosterone and of Cobalt on Erythropoietin Production by Anephric Rats. *J. Lab. Clin. Med., 74: 623-629, Oct. 1969.*

The effects of cobalt and of testosterone on the plasma erythropoietin titers of anephric hypoxic rats were studied. Whereas cobalt did significantly enhance erythropoietin production in these animals, testosterone had no detectable effect.

Konietzko, N. and Carton, R. W.: Diffusion Defect in Patients with Scleroderma. *Beitr. Klin. Tuberk. 139: 196-205, 1969.*

Clinical, reontgenological, and physiological findings are reported in 20 patients with systemic sclerosis examined between 1962 and 1968. Seven patients showed evidence of a restrictive ventilatory defect; two patients showed evidence of obstruction. Decreased CO-single-breath-diffusion-capacity of the lung was the most frequently observed physiological abnormality. The different mechanisms involved in this test are discussed. Clinical symptoms proved to be of minor importance for the diagnosis of parenchymal involvement of the lung. The best correlation was found between the X-ray of the lung and the diffusion capacity.

Garron, David C. and Van der Stoep, Laima R.: Personality and Intelligence in Turner's Syndrome. *Arch. Gen. Psychiat., 21: 339-346, Sept. 1969.*

The sex chromosome abnormality, sexual immaturity, and female habitus of persons with Turner's syndrome raise questions about the influence of genetic, somatic, and social factors on the development of both personality and intelligence. Reports indicate that erotic drive is low but heterosexually appropriate, that interests and attitudes are feminine, and that the incidence of emotional disorder is low despite concern with short stature and obesity. Mental retardation is not invariably associated with Turner's syndrome. A reanalysis of published data indicates that mental retardation may not even be more frequent among such persons than among the normal population. Thus, persons with the syndrome are to be found throughout the intellectual range. Non-verbal abilities are slightly impaired, however, relative to verbal abilities. It may be suggested, therefore, that persons with Turner's syndrome, particularly school-age children, be given intelligence tests which are not limited to verbal skills.

Koestline, W. Charles and Dent, Oran B.: Verbal Mediation in the Wais Digit Symbol Subtest. *Psychol. Rep., 25: 377-378, 1969.*

It has been speculated that a verbal factor is involved in WAIS Digit Symbol (DS) subtest performance. Sixty subjects were supplied with familiar verbal labels, unfamiliar verbal labels or no labels for each of the symbols of the DS subtest. Pre- and post-DS performance scores and a digit-symbol association score were compared among groups. More digit-symbol associations were recalled after learning familiar than unfamiliar labels, but no differences wre found in DS performance. These results were interpreted as minimizing the possibility of a verbal loading and arguing for a psychomotor interpretation of DS performance.

Eisenstein, Reuben, Ellis, Hellen, and Rosato, Jacqueline: *In Vitro* Studies of Vitamin D-induced Aortic Calcification. *Proc. Soc. Exp. Biol. Med., 132: 58-62, Oct. 1969.*

Arterial segments incubated in a culture system containing serum from rats with hypervitaminosis D accumulate more calcium than segments incubated in a system containing normal rat serum. If such arterial segments are incubated in serum, they accumulate more calcium from normal serum than rachitic serum and more from serum from rats with hypervitaminosis than from normal serum. Addition of either vitamin D or calcium alone to normal serum does not result in excess calcium accumulation by arteries incubated in such serum, but addition of both does. Different aortic segments accumulate different amounts of calcium, apparently in relation to the proportion of elastic tissue in the segments.

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ACUTE IMPROVEMENT BY CHEMICAL MEANS OF VISUAL AND OCULOMOTOR SIGNS IN MULTIPLE SCLEROSIS: PRELIMINARY COMMUNICATION

Floyd A. Davis
Frank O. Becker
Joel A. Michael
Eric Sorenson

INTRODUCTION

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system in which it is believed that conduction in diseased axons is slowed or blocked.[1] Small changes in body temperature are known to produce acute changes in the signs and symptoms of MS patients, with heating[2] causing a worsening, and cooling[3] an improvement. These changes are reversible, and last only so long as the temperature changes are maintained. Perhaps the chief significance of these findings is the fact that the pathophysiologic changes which cause the signs and symptoms of MS can be acutely altered without concommitant structural changes. This suggests that conduction in demyelinated axons can be altered and perhaps improved by exogenous factors.

Recent work in experimental animals[4-6] supports the hypothesis that the effect of elevated temperature on demyelinated or damaged nerves is to decrease the safety factor which is defined as the ratio of action current available for propagating the impulse to the minimum amount of current needed to just maintain conduction;[7] this ratio reflects the net effect of many variables involved in the maintenance of conduction. A decrease in the safety factor would be expected to impair conduction. Conversely, it has been shown that cooling restores conduction in damaged nerves. This suggests that other procedures which increase the safety factor might also be useful in counteracting the effects of demyelinization. This suggestion was indirectly borne out by the finding[5,6] that reduction of the calcium concentration in the external medium bathing a damaged nerve (thereby increasing excitability and hence the safety factor) improves conduction and counteracts impaired conduction resulting from temperature elevation.

From the Department of Neurology, Division of Medicine, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois

This work was supported in part by a grant from the Morris Multiple Sclerosis Research Fund and Presbyterian-St. Luke's Hospital Research and Educational Committee Grant No. 37156. Some of the equipment used in this study was provided by a grant from Mr. F. Norwood Bard to the Department of Bio-Medical Engineering.

Floyd A. Davis, M.D., Assistant Attending Neurologist
Frank O. Becker, M.D., Assistant Attending Physician in the Section of Endocrinology and Metabolism
Joel A. Michael, Ph.D., Assistant Attending Bioengineer, Department of Bio-Medical Engineering
Eric Sorenson, M.D., Resident, Department of Ophthalmology

For this reason it appeared worthwhile to test the effects on MS patients of procedures believed to produce a reduction in ionized calcium. It was hoped that such procedures might mimic the beneficial effects of hypothermia. The procedures selected were hyperventilation (decreased pCO_2 → alkalosis → decreased ionized calcium), infusion of sodium bicarbonate (alkalosis → decrease in ionized calcium), and infusion of disodium ethylene diamine tetraacetate (EDTA) which produces a Ca-EDTA complex, thus reducing the concentration of the ionized form. In order to obtain quantitative, objective measurements of the response of patients to these procedures it was decided to monitor lateral gaze nystagmus and visual field scotomas, both common findings in MS.

METHODS AND MATERIALS

Eight patients have been studied, two male and six female. Their ages range from 21 to 47, and the duration of their disease from seven months to five years. All have lateral gaze nystagmus and/or scotomas.

The movements of each eye were monitored independently using an electro-oculographic technique[8] employing electrodes placed at the canthi of the eyes and AC- or DC-coupled amplifiers. In all experiments the frequency of nystagmus (beats/second) was the parameter measured. Patients were seated in front of a perimeter equipped with three fixation lights, one at the center, the others to right and left of center. The latter two were positioned to produce the maximal nystagmus compatible with the ability to comfortably maintain fixation. The lights were alternately turned ON and OFF, and the patient was told on which light to fix. In this way, a pattern of alternating right and left lateral gaze nystagmus was elicited.

Visual fields were mapped, and the location and extent of the scotomas determined in the conventional manner using a Bausch & Lomb Autoplot Tangent Screen with standard red and white targets.

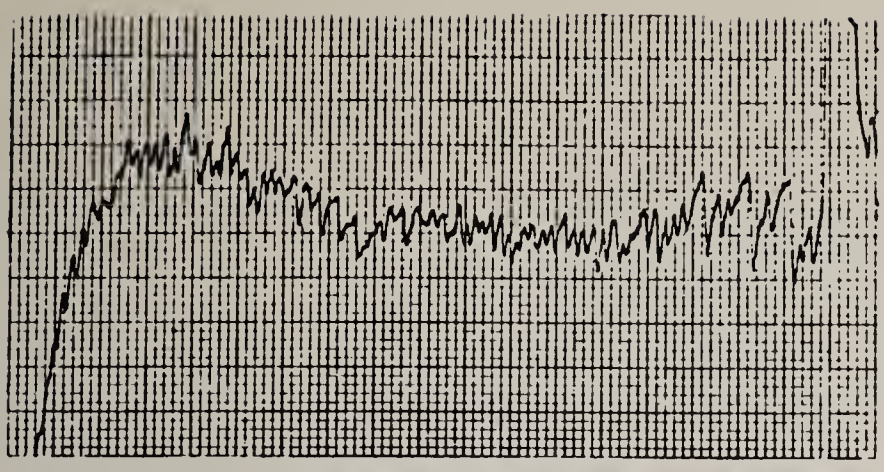
Experiments were carried out in the following manner. Control responses were obtained prior to the beginning of any experimental procedure. The particular procedure to be tested was then begun, and responses were recorded throughout its duration. Finally, recovery was monitored by observing responses for a period of time after cessation of the procedure.

The three procedures tested were hyperventilation for two to three minutes, intravenous infusion of 500-1000 ml of five per cent sodium bicarbonate solution (15-25 ml/min), and intravenous infusion of Na_2EDTA solution (3 grams in 1000 ml. of normal saline given over a period of 50-80 minutes).

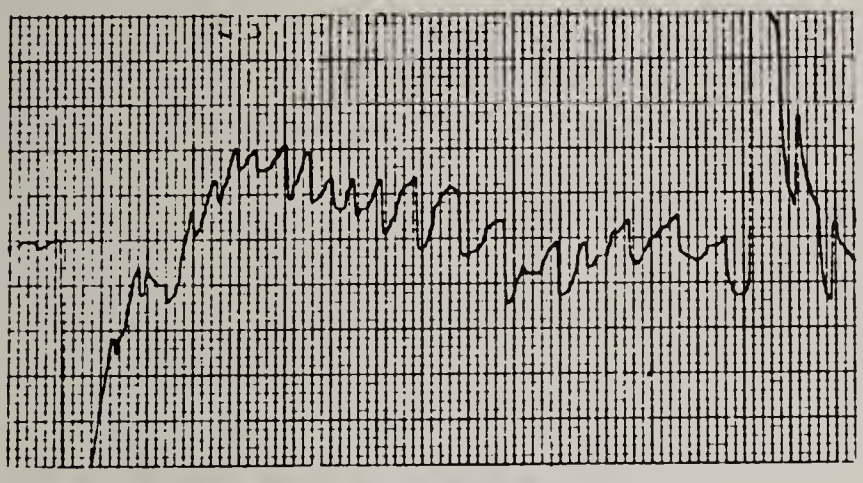
RESULTS

Nystagmus. In all patients, each of the procedures tested caused a reduction in nystagmus frequency and, in some instances, a complete cessation of nystagmus, as compared with control determinations. This change was completely reversible in that, following cessation of hyperventilation or either infusion, there was a gradual return of nystagmus frequency to its control level. Samples of the eye-movement records obtained from a patient before, during, and after hyperventilation are seen in Fig. 1. Tables 1 and 2 summarize the results in all patients. Note that these effects were readily reproducible in the same patients over extended periods of time—in one case, with repeated testing, for two and one-half months. All patients tested responded to the experimental procedures.

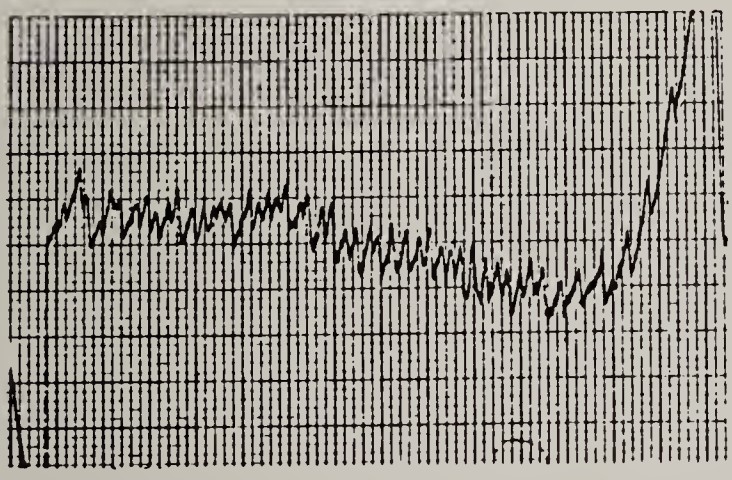
Visual Field Defects (Scotomas). Bicarbonate and EDTA infusions each produced marked reduction in the size of scotomas in the three patients tested. Fig. 2 shows visual fields determined at various times during a bicarbonate infusion experiment. Note the shrinking of the scotoma during the infusion and its gradual return following the cessation of infusion. These results are typical of those obtained with the other two patients, one



PRE-HV



HV($2\frac{1}{2}$ MIN)



POST - HV($1\frac{1}{2}$ MIN)

Fig. 1—Eye movement records from a patient before, during, and after hyperventilation. Note the marked decrease in nystagmus frequency during hyperventilation and the rapid return to control levels following cessation of rapid breathing.

infused with bicarbonate and one with EDTA.

Because of the short-lived effect of hyperventilation and the time necessary for plotting visual fields, the effects of hyperventilation on scotomas was not attempted.

Control Experiments. The marked sensitivity of MS patients to changes in body temperature raised the question of whether hyperventilation or the infusion of room-temperature solutions could be cooling the patients sufficiently to produce improvement in their signs. This possibility was ruled out by having the patient hyperventilate air that had been heated to 101°F. Under this condition it is unlikely that hyperventilation could produce a cooling effect; nevertheless, a reduction in nystagmus frequency occurred. In analogous experiments, heated solutions (99°F) of sodium bicarbonate were used. With these, nystagmus and scotomas still showed the typical improvement. Finally, infusions of normal saline were given to see if this alone could in some way produce an effect; none was noted.

TABLE 1

SUMMARY OF EFFECTS OF HYPERVENTILATION ON ALL PATIENTS

Subject	Date	Nystagmus Frequency (Control)	Minimum Frequency During Hyperventilation	Per Cent Control	Time to Minimum Frequency	Time to Return
R.W.	8/8	2.66 beats/sec	1.33 beats/sec	50.0	0'25"	
	8/22	2.83	1.75	61.7	2'15"	1'00"
		3.16	1.75	55.0	1'37"	1'15"
	8/29	3.57	1.41	39.5	2'30"	1'30"
	10/10	3.00	1.33	39.0	2'45"	5'00"
	10/24	2.25	1.33	59.0	2'00"	2'00"
E.M.	8/15	2.33	0.00	00.0	1'00"	2'00"
	10/3	2.21	1.50	67.9	3'00"	2'30"
C.N.	11/28	1.88	0.60	32.0	2'45"	1'45"
	12/5	2.58	0.90	34.8	1'00"	3'00"
T.A.	8/1	(CLINICAL IMPROVEMENT OF NYSTAGMUS OBSERVED)*				
D.R.	8/1	(CLINICAL IMPROVEMENT OF NYSTAGMUS OBSERVED)*				
	9/12	(CLINICAL IMPROVEMENT OF NYSTAGMUS OBSERVED)*				
M.F.	11/14	(CLINICAL IMPROVEMENT OF NYSTAGMUS OBSERVED)*				

*Technical difficulties in obtaining eye movement records permitted only clinical observations.

The question was also raised as to whether it was possible that the infusions were acting through a change in serum osmolarity. However, when serum osmolarity was increased, with 10% glucose infusions, to levels comparable to those attained with experimental infusions, no changes occurred in the scotomas.

Thus, it seems reasonable to conclude that the improvement in nystagmus and visual field scotomas produced by hyperventilation, sodium bicarbonate, and EDTA infusions is not due to either artifactual temperature effects or osmolarity changes.

DISCUSSION

The experiments reported here demonstrate that two of the common signs in multiple sclerosis, lateral gaze nystagmus and central scotomas, can be modified by hyperventilation, infusion of sodium bicarbonate, and infusion of Na_2EDTA. With all three procedures, nystagmus frequency and scotoma size are reduced. These effects are transient,

TABLE 2

SUMMARY OF EFFECTS OF BICARBONATE AND Na_2EDTA INFUSION ON ALL PATIENTS TESTED

Subject	Date	Nystagmus Frequency (Control)	Minimal Nystagmus Frequency During Infusion: Bicarbonate	Na_2EDTA	Per Cent Control
R.W.	9/5	3.59 beats/sec	1.00 beats/sec		28
	8/29	2.67		1.17	45
C.N.	11/28	1.84	1.05		57
	12/5	2.50		1.10	44
E.M.	10/3	2.59	1.83		71

lasting only as long as the procedures are continued.

Control experiments have eliminated the possibility that these effects are the result of non-specific cooling effects due to infusion of room-temperature solutions. Osmolarity effects appear unlikely since no clinical changes occurred with control hypertonic glucose infusions producing comparable osmolarity changes. We are thus left to conclude that the observed changes in MS signs are due to some chemical change in the environment of the nervous system, produced by the three procedures.

Furthermore, it can be concluded that the effects are central rather than peripheral. Certainly in the case of changes in visual field defects, no other conclusion is as plausible; scotomas in MS are thought to be due solely to lesions of the optic nerve, a central nervous system tract. The locus at which the three tested procedures affect nystagmus frequency is more difficult to determine; both a central effect at the brain-stem level and a peripheral neuro muscular effect are possible. However, the findings with scotomas strongly support the idea that with nystagmus the effect is probably a central one, or at least predominately central.

The clinical importance of this report lies in the demonstration that signs in MS can be favorably altered by chemical means. This is not to say that these three procedures constitute a form of therapy. Rather, their successful employment here

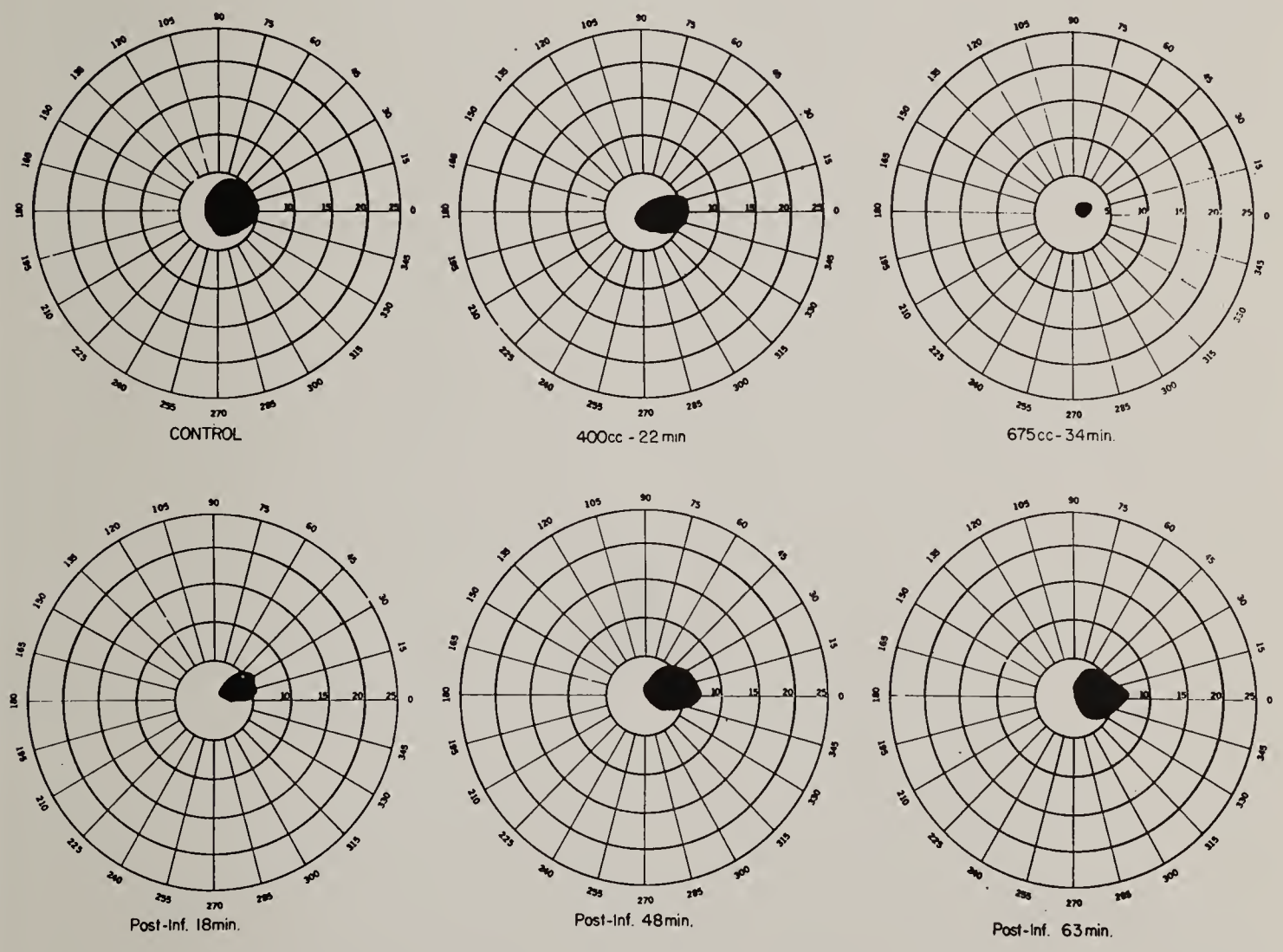


Fig. 2—Visual fields determined with a one-millimeter white target before, during, and after infusion of sodium bicarbonate. The size of the scotoma slowly decreases during the infusion and then slowly increases, approaching control size after cessation of infusion.

points to a new approach to the investigation of pharmacological symptomatic therapy for MS. In addition, the animal models on which the initial concepts were developed[5–7] would appear to be useful in the screening of drugs for potential use with MS patients.

It should be noted that the exact mode of action of the three procedures employed is still not completely clear. It was initially hypothesized that the results obtained would be the consequence of a state of lowered ionized calcium, and in the case of EDTA this appears to be the only explanation. However, it is known that changes in pCO_2 can directly affect the excitability of neural membranes[9] and it is possible that with hyperventilation, such an additional effect applies. In any event, the phenomenon itself is clear and has important implications. Further experimentation will be guided by the hypothesis that drugs tending to increase the conduction safety factor may have a beneficial effect on MS patients.

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THE SERUM PROTEIN-BOUND IODINE (PBI) IN ACUTE MYOCARDIAL INFARCTION: A PITFALL IN CLINICAL INVESTIGATION

Robert Hedger

A chance observation suggested that the serum protein-bound iodine (PBI) became elevated in patients after an acute myocardial infarction. Data compiled from 23 patients admitted for chest pain, in what appeared to be a controlled study, documented this relationship. Shortly after the completion of the study, another explanation for the phenomenon emerged[1] which prompted reevaluation of the data and further investigation. The data are presented in chronological order to emphasize the hazards in interpretation of clinical studies.

Materials and Methods

Thirty patients, admitted to Presbyterian-St. Luke's Hospital for severe chest pain during a six-week period, were the subjects of the study. Each patient was seen personally and if the history suggested any exposure, therapeutic or diagnostic, to iodinated compounds, the patient was excluded from the study. Twenty-three patients met these criteria. A serum protein-bound iodine, done by a modified Technicon method,[2] a serum thyroxine (Murphy-Pattee), adapted after Murphy,[3] using hemoglobin-coated charcoal as the absorbent, and a T-3 uptake[4] were obtained on the first and second hospital day. Following this, a PBI and serum thyroxine (Murphy-Pattee) were obtained twice a week. If chest pain requiring analgesics occurred after the first 48 hours, the PBI and serum thyroxine were obtained on the next two days and then twice a week.

From the Department of Medicine, Division of Medicine, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Robert Hedger, M.D., Research Fellow in the Renal and Nutrition Section, Department of Medicine

The diagnosis of acute myocardial infarction was based on the history of severe chest pain, ST elevation with evolution on serial electrocardiograms, elevation of the serum glutamate-oxaloacetate transaminase (SGOT) and lactic dehydrogenase (LDH), or by necropsy. Two patients had chest pain, SGOT and LDH elevation, and non-diagnostic electrocardiographic changes. All patients were treated with strict bedrest, oxygen, analgesics, and sedation. Diuretics, intravenous fluids, digoxin, and antiarrhythmic drugs were used if clinically indicated. Most patients received heparin and then coumadin. The diagnosis of angina pectoris was based on an abnormal non-specific electro-cardiogram with chest pain relieved by nitroglycerin. The diagnosis of familial idiopathic cardiac hypertrophy was made at autopsy. The diagnosis of pneumonia was based on gram positive *cocci* in the sputum and radiologic signs of a pulmonary infiltrate. A patient with pain, transient hyperbilirubinemia, and leukocytosis was diagnosed as having acute cholecystitis. The SGOT and LDH were done according to Henry[5] at 32 degrees centigrade.

Results

Table 1 lists all of the patients and the pertinent data of the initial study. There were 19 males and 4 females, ranging in age from 40 to 87 years. The PBI became elevated in eighty-eight per cent of the patients with acute myocardial infarction. The average elevation was 12.3 μmg/100ml (ranging from 7.8 to 14.2). In the survivors the PBI became elevated an average of 12.3 μgm/100ml (ranging from 7.8 to 14.2), reaching a peak in 3.5 days (ranging from 2.0 to 7.0), and returning to normal by the 15th day (ranging from 10 to 30). Using 5.4 μmg/100ml as normal, the PBI in the patients that expired became elevated an average of 13.6 μgm/100ml (ranging from 11.2 to 14.6), and peaked in 6.6 days (ranging from 4 to 10). The PBI became elevated in three of six patients with diagnoses other than myocardial infarction.

Fig. 1 is a graph of the course of a patient characteristic of the ten patients that survived. There was a rapid elevation and then a slow regression of the PBI.

The patient *J.O.* was initially diagnosed as having pre-infarction angina. Repeated SGOT's and PBI's were normal during the first five hospital days. On day six he had severe chest pain, and the following day the SGOT had risen from 41 to 82 units, and the PBI from 3.3 to 15.0 μgm/100ml. The electrocardiogram showed a shift in axis but was not

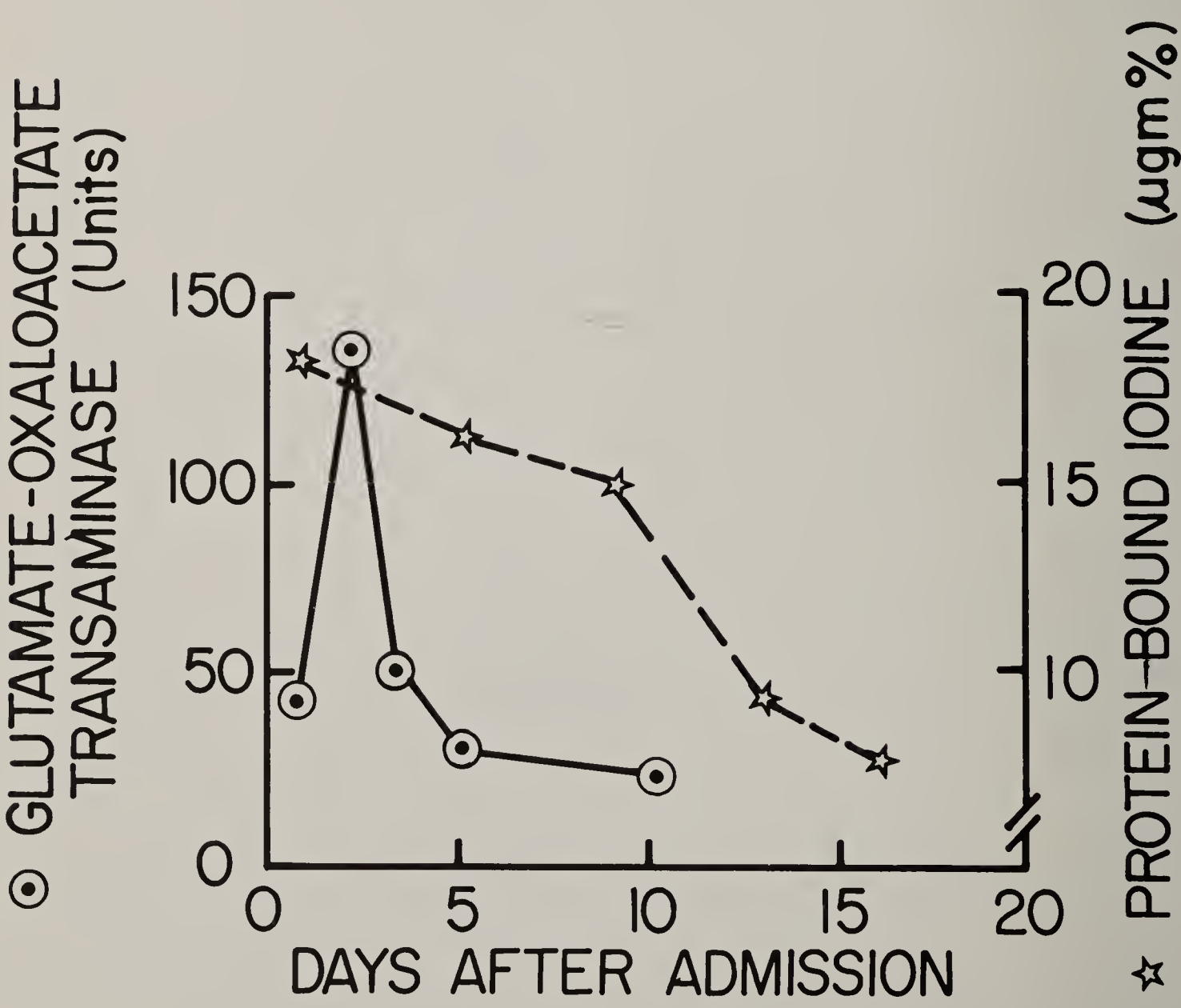

Fig. 1—The serum glutamate-oxaloacetate transaminase (0) and protein-bound iodine (△) levels in patient GF. Characteristic of the group of patients that survived following an acute myocardial infarction.

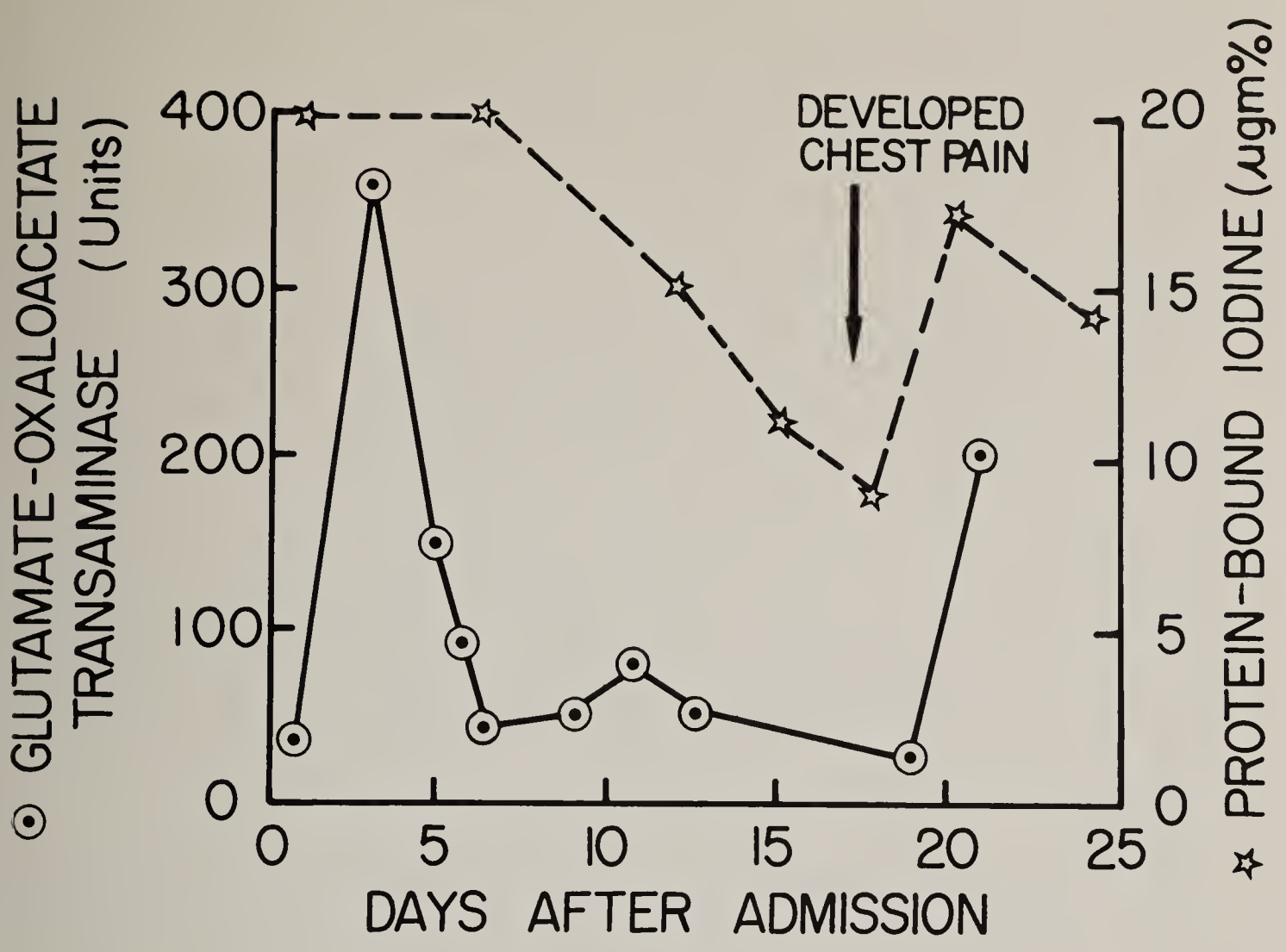

Fig. 2—The serum glutamate-oxaloacetate transaminase (0) and protein-bound iodine (△) levels in patient A.S. Characteristic of the group of patients that expired following an acute myocardial infarction.

diagnostic of an acute myocardial infarction.

Fig. 2 summarizes the course of one patient who expired. The PBI remained elevated at 20 μgm/100ml for six to seven days, and then began to decline. About the 20th hospital day he had recurrent chest pain with a cardiac arrest. He responded to therapy initially but then expired on the 25th day. Autopsy showed acute and organizing myocardial infarctions. *G.S.* also had recurrent chest pain without electrocardiogram or enzyme changes, but the PBI became elevated a second time. At autopsy two myocardial infarctions were present, morphologically in concert with the chest pain and PBI elevations.

The serum thyroxine (Murphy-Pattee) tended to drift upward as the PBI came down, but remained in the normal range. The correlation coefficient for the PBI and serum thyroxine was $r_6-[_{76}] = 0.3533$. $r = 0$ is <0.01.

The T-3 uptake was normal in all cases.

From the data presented, the PBI became elevated in 88% of the patients with myocardial infarction and followed a fairly predictable pattern. The PBI elevation in the survivors appeared rapidly, and by the fourth day was beginning the downward trend. The PBI elevation in patients who expired appeared rapidly and remained elevated for a longer time, starting to regress at about one week. All of the patients that manifested a PBI of 20 at any time during observation developed significant cardiac decompensation.

A similar study by Azar[6] was in direct conflict with the above data, but the discrepancy might have been explained on the basis of the methods used to measure the PBI.[7] Therefore, at the completion of the study there seemed to

TABLE 1

THE DIAGNOSIS, SERUM GLUTAMATE-OXALOACETATE TRANSAMINASE, AND THYROID FUNCTION VALUES IN 23 PATIENTS ADMITTED TO HOSPITAL FOR SUSPECTED MYOCARDIAL INFARCTION

Diagnosis, Age & Sex	Protein-Bound Iodine* (μgm/100ml) Range	Day Following Chest Pain PBI* Elevated	Day Following Chest Pain PBI* Returned to Normal	Total Serum Thyroxine* (μgm/100ml) Range	Triiodothyronine* Uptake % Range	Glutamate Oxaloacetate Transaminase* (units) Range	Day Following Chest Pain GOT* Elevated
Myocardial Infarction:							
J.K. 87F	20+ -12.8	1			39.9	310-190	3
A.S. 68M	20+ - 9.0	1		8.2-7.2	40.1-37.3	360- 28	3
R.W. 67M	20+ - 5.8	2	30	8.6-2.9	31.9	160- 37	2
P.R. 74M	19.8-14.4	2		8.5-6.8	42.0-37.0	105- 42	5
G.S. 54M	19.0- 7.7	2	24	6.1-3.9	39.1-38.5	625- 40	2
G.F. 45M	18.6- 5.7	2	12	7.1-5.0	33.7-32.4	135- 32	2
J.N. 46M	18.4- 5.9	2	15	6.5-5.4	40.0-37.0	162- 20	2
H.G. 63M	17.9- 5.1	2	17	5.6-3.8	32.9-32.4	530- 41	1
F.W. 68M	17.4- 5.1	7	17	4.5-2.5		174- 27	1
G.G. 59F	16.7- 8.9	1	13	7.1-5.0	39.2-37.7	315-130	2
R.B. 60M	16.5- 6.4	2	13	5.8-4.0		310- 27	1

E.S. 45M	15.4- 9.2	2					
B.O. 47M	6.0- 4.0	Never	Always	6.7-2.5		88- 15	2
M.D. 82F	6.3- 6.4	Never	Always	5.9-5.1	42.3-41.3	340-100	1
Pneumonia:							
A.B. 60M	14.0- 8.8	2		9.1-	38.1-36.3	36- 30	Never
Angina Pectoris							
E.T. 67F	11.0- 8.4	3	Diagnostic Dye Day 15	5.1-4.0	41.0-35.7	33- 25	Never
H.K. 70M	7.0- 5.4	Never	Always	7.4-5.5	39.7-	20- 18	Never
H.K. 70M	6.1- 5.0	Never	Always	5.1-3.9	38.8-36.0	20- 19	Never
Familial Idiopathic Cardiac Hypertrophy:							
E.J. 56M	14.4- 8.7	3	Never	7.8-3.9	40.1-	340- 32	4
Cholecystitis:							
R.G. 40M	5.2- 4.0	Never	Always	6.5-4.6	37.6-	29- 16	Never

*NORMAL RANGES: Protein-bound iodine (PBI) 3.4-7.4 μgm/100ml
Total serum thyroxine 3.7-7.0 μgm/100 ml
Triiodothyronine uptake 30.0-45%
Glutamate-oxaloacetate transaminase (GOT) 10-50 units

be a direct correlation between acute myocardial infarction and an elevation in the PBI.

No serious challenges to the validity of the study emerged when these data were presented at a research seminar of the Department of Medicine. However, at this point, Simbari's[1] brief report of distortion of PBI determination during coronary care came to our attention. This author found that the Bard Intracath—the type used in all the patients with elevated PBI—contained between 20% and 50% iodide! He identified the compound as an aliphatic iodide having the ability to bind to serum proteins. Further investigations were therefore instituted.

Two patients with acute myocardial infarctions were treated as the others had been, but the Bard Intracath was not used. The PBI remained normal for five days. On the afternoon of the fifth day, a Bard Intracath was put in place, and the following day the PBI became elevated in both subjects. The pattern of elevation and regression was very similar to the other patients studied.

Five patients, without cardiac disease, who received intravenous fluids via a Bard Intracath were also studied. One patient had carcinoma of the breast, one had acute pancreatitis, and three had acute gastrointestinal bleeding. The PBI became elevated the day after the Bard Intracath was put in place in four of the five patients. The PBI elevation was not of the magnitude noted in the patients with an acute myocardial infarction, but the pattern was similar. The average increase was 7.0 μgm/100ml. Two patients had a second Bard Intracath put in place, and in each case the PBI became elevated again. The second elevation was 2 to 3 μgm/100ml. The PBI returned to normal in four to seven days.

These studies thus verified an artificial elevation of the PBI produced by the Bard Intracath and clearly pointed to iodine contamination in the initial study. The elevated PBI during an acute myocardial infarction merely reflects the use of a Bard Intracath in these patients, without relation to the basic pathlogic process.

A few practical points about the use of the Intracath can be made. It produces an elevation of the PBI in the serum of 50% to 88% of patients. The degree of increase is variable and can range from 2 to over 15 μgm/100ml. Usually five to ten days must elapse after an Intracath is put in place before the PBI is again valid. However, if liver function is deranged it may take as long as 30 days before the PBI returns to normal. This was exemplified by the prolonged elevations in patients with congestive heart failure and abnormal liver function tests. If more than one Intracath is used, an additive effect can be expected. The Bard Intracath does not affect the value of the serum thyroxine (Murphy-Pattee) or T-3 resin uptake.

SUMMARY

Elevation of PBI in a series of 23 patients treated for acute myocardial infarction was puzzling. Information from the literature prompted clinical observations which indicate that the commonly used Bard Intracath was the probable source of iodine contamination leading to spurious results.

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DEXAMETHASONE SUPPRESSION OF URINARY 17-HYDROXYCORTICOIDS IN A PATIENT WITH AN ACTH-PRODUCING BRONCHIAL ADENOMA

Gretajo Northrop
David Baldwin
L. Penfield Faber
Theodore B. Schwartz

INTRODUCTION

In 1928, Brown[1] described a patient with hirsutism, truncal obesity, pedal edema, pigmentation, hypertension, and hyperglycemia. Post-mortem examination revealed enlargement of the pituitary and adrenal glands and an "oat-cell" carcinoma, measuring 1 cm in diameter in the lung. A number of reports concerning patients with Cushing's syndrome and pulmonary cancer have subsequently been reported; however, Cohen, *et al.* in 1960[2] were the first to report the association of Cushing's syndrome and bronchial adenoma. Christy in 1961[3] demonstrated elevation of corticotrophic activity in the plasma of two patients; one patient had a bronchogenic carcinoma and the other patient had a bronchial adenoma. However, it remained for Meador, *et al.* in 1962[4] to show that non-endocrine tumors could contain a material similar to, if not identical with, ACTH.

Although such patients[5] have been reported with increasing frequency, bronchial adenomas as the source of the excessive ACTH remain a rarity.

The patient to be presented had Cushing's syndrome and an ACTH-containing bronchial adenoma of mixed histological type. Unexpectedly, suppression of urinary 17-keto and hydroxysteroids followed administration of 8 mg of dexamethasone[6] to this patient. A discussion of this and other biochemical abnormalities demonstrated by patients sharing this unique type of neoplasm will be presented.

From the Section of Endocrinology and Metabolism, Division of Medicine, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Gretajo Northrop, M.D., Ph.D., Research fellow in the Section of Endocrinology and Metabolism; recipient of Public Health Service Special Research Fellowship #1 F3-AM-40, 887-01 (d)
David Baldwin, M.D., Attending Physician
L. Penfield Faber, M.D., Associate Surgeon and Director of the Section of Thoracic Surgery
Theodore B. Schwartz, M.D., Attending Physician and Director of the Section of Endocrinology and Metabolism

CASE REPORT

W. M. E. (P.S.L.H. #817575), a 44-year-old, white, sales manager, was admitted to Presbyterian-St. Luke's Hospital on March 1, 1967 for evaluation of a one-year history of weakness and paresthesias in his extremities; a 30-pound gain in weight; and episodes, increasing both in frequency and severity, of edema. The patient was also aware of a personality change, *i.e.*, recently he had become increasingly introspective and

irritable. Following a workup at another hospital in 1965, he was told that he had high blood pressure, an elevated blood sugar, and an increased amount of protein in his cerebrospinal fluid. He was discharged with a diagnosis of multiple sclerosis. His family history was negative for tuberculosis, cancer, diabetes, gout, collagen disease, renal and cardiovascular disease. He admitted consumption of generous amounts of ethanol and he smoked one pack of cigarettes per day.

On physical examination he was noted to be a mildly plethoric, centripetally obese male, with labile moods. His blood pressure was 160/100 mm of Hg; his heart rate was regular with a rate of 80 beats per minute. He weighed 211 pounds and was 5 feet, 11 inches tall. Examination of the skin revealed a mildly plethoric facies, and a few bluish-red abdominal striae without evidence of hyperpigmentation or atrophy. Examination of the chest and abdomen were unremarkable; no cardiomegaly, hepatosplenomegaly or abdominal masses were noted. Minimal bilateral pedal edema was present. The only abnormality observed during neurological examination was mild bilateral muscle weakness with slight atrophy, which involved predominantly the proximal groups of his lower extremities. There were no demonstrable abnormalities in his reflexes or sensory perception.

Initial laboratory studies revealed normal hematologic and urine analyses. Serum electrolyte concentrations were: sodium 142, potassium 4.8, chloride 102, carbon dioxide 29.9 mEq/L, and calcium 8.2 and phosphorous 3.2 mg/100ml. Because of the previous history of carbohydrate intolerance and of increased protein in the cerebrospinal fluid, further diagnostic tests were performed. The results of an oral glucose tolerance test were: fasting 85, one-half hour 150, one hour 170, two hours 113, three hours 55, four hours 70, and 5 hours 76 mg/100ml. The results of electrophoresis on a sample of cerebrospinal fluid reported in mg/100ml were: total protein 140, albumin 82, and the globulins $alpha_1$ 12, $alpha_2$ 12, beta 19, and gamma 15. A Wasserman test performed on the cerebrospinal fluid was negative, and the colloidal gold curve was 1122110000. In view of the history of generous ethanol ingestion, a liver profile was obtained. Serum electrophoresis expressed in g/100ml showed: total protein 6.8; albumin 3.9; and the globulins, $alpha_1$ 0.3, $alpha_2$ 0.9, beta 0.8, and gamma 0.9. Additional studies, all within normal limits, included: bromsulphalein 3.1%, prothrombin first stage 100%, alkaline phosphatase 1.9 Bessey-Lowry units, serum glutamic-pyruvic transaminase 20, and isocitric dehydrogenase 140 units/ml. Studies to evaluate his muscle weakness were obtained: lactic dehydrogenase 470, aldolase 3, and phosphocreatine kinase 5 units/ml of serum. The protein-bound iodine was 5.4 μg/ml. Because of his truncal obesity, edema, elevated blood pressure, muscle weakness and abdominal striae, estimation of steroid concentrations in the plasma and urine were obtained. These and additional studies of adrenal cortical stimulation and suppression are summarized in Tables 1 and 2.

Roentgenograms of the patient's chest taken in 1965 were obtained from his previous hospital admission for comparison with those taken in 1967 (Fig. 1). The laminogram taken in 1967 (Fig. 2) clearly delineates the 3x3½-centimeter mass in the right cardiophrenic angle, which had been reported as probably a fat pad. Additional studies revealed a normal-sized sella, generalized bony demineralization in the spine and a normal intravenous pyelogram and transfemoral aortogram.

On March 23, 1968, the patient had a right thoracotomy. The right middle lobe of lung was removed; it contained a tumor which was discrete and had no connection with the bronchus on gross examination. Regional lymph nodes showed no evidence of metastatic disease. His post-operative course was complicated by congestive heart failure and the presence of copious amounts of secretions from the respiratory tract, re-

TABLE 1

RESULTS OF STEROID ANALYSES ON PLASMA AND URINE

TREATMENTS and ASSAY METHODS	PLASMA	URINE	
	Cortisol (μg/100 ml)	17-Hydroxysteroids (mg/24 hrs)	17-Ketosteroids (mg/24 hrs)
Method of Assay	Fluorimetric[7]	Colorimetric[8]	Colorimetric[9]
Laboratory Normals (PSLH)	6-25	3-12	9-18 males
Pre-surgery			
Baseline	67	19.5	20.6
Dexamethasone 1 mg	53		
2 mg		16.1	16.0
8 mg		6.3	7.5
Post-surgery			
Day *Drugs*			
1 Hydrocortisone sodium succinate	55		
2 Prednisone			
3 Prednisone & spironolactone	50		
4 Spironolactone	84		
5 Spironolactone	110		
6 Spironolactone	106		
7 Spironolactone	64		
8			
9			
10			
11 Spironolactone	60	4.8	7.8
12 Spironolactone		2.0	4.5
13			
14		2.5	6.1
15	16		
16		1.2	2.0
17		1.2	4.9
18	8	2.2	5.2
19		1.9	4.2
20	9	2.6	4.9
21	15		
22	12	2.8	5.0

*False elevation of plasma cortisol as determined by the fluorimetric[7] assay has been reported.[10] Simultaneous determinations of urinary steroids by colorimetric[8,9] procedures on the eleventh post-operative day did not reflect the spironolactone-induced false elevation of plasma cortisol concentration.

sulting in bilateral pneumonia. Cultures from tracheal aspirations were not diagnostic of specific pathogenic organisms; however, following administration of digoxin and improvement in tracheal toilet through a tracheostomy, his physical condition rapidly improved. He was discharged on April 16, 1967 and was requested to continue his daily maintenance dose of digoxin.

On April 19, 1968, the patient was readmitted with a three-day history of progressive anorexia, lethargy, and right upper abdominal tenderness. Laboratory data supported the clinical impression of acute cholecystitis. He was managed conservatively and discharged on May 4, 1967. During this admission his plasma cortisol concentration was 15.9 μg/100ml and 24-hour urinary 17-hydroxy- and 17-ketosteroids were 2.7 and 2.5 mg respectively. Because of the low urinary steroid levels and the stress of his illness the patient was placed on 12.5 mg of

cortisone acetate twice a day. (The patient refused an ACTH infusion test to evaluate adrenal reserve).

Following the last hospitalization, he continued to feel depressed and despondent. He developed polyarthralgias and some weakness, and it was felt that he had either rheumatoid arthritis, which had been suppressed by the hypercorticism existing prior to surgery, or more likely, that he was showing symptoms of steroid withdrawal.[11] During the next few months digoxin was discontinued, and he was placed on 10 mg of prednisone per day. He remained psychologically and physiologically asymptomatic, working full time until August, 1968, when he had another episode of acute cholecystitis.

DISCUSSION

The roentgenograms of the chest for 1965 and 1967 (Fig. 1) are presented for comparison. The abnormality in the right lower lung field was initially interpreted as probably a fat pad. Mediastinal widening and the appearance of a right epicardial fat pad have recently been reported in patients who were taking exogenous glucocorticoids. After discontinuing the exogenous steroids the chest X-rays in these patients became normal.[12] In the laminogram presented in Fig. 2, the density in the right lung field of our patient is clearly delineated as a solid, noncalcified mass.

Photomicrographs are presented in Fig. 3 of two adjacent areas of this tumor. The typical homogeneous carcinoid pattern of the bronchial adenoma can be observed in 3A. On the other hand, 3B shows a portion of the tumor which has many characteristics of the cylindroid type of bronchial adenoma. To our knowledge, this is the first report of an ACTH-secreting bronchial adenoma with a cylindromatous component. The other 18 tumors reported were either unclassified or carcinoid in type.

Elevation of the protein in the cere-

TABLE 2

Results of Electrolyte, Adrenal Suppression and Stimulation Data Obtained from the Literature in Patients with Bronchial Adenomas and Cushing's Syndrome. (Numbers refer to the references at the end of the article.)

Literature Reference Nos.	Case	Hypokalemia	ACTH Stimulation	Dexamethasone Suppression
2	1	absent	0	0
2	2	0	0	0
3	B.C.	0	present	0
17		present	present	±*
18	3	0	0	0
19		present	present	not suppressed
16	526	present	0	0
20		0	0	0
21		0	0	not suppressed
22		present	present	not suppressed
23	6	absent	present	not suppressed
24		present	present	not suppressed
25		present	present	not suppressed
26		present	0	suppressed
27	1	absent	present	suppressed
27	2	present	present	suppressed
28		present	present	suppressed
29		present	0	suppressed
Our Patient		absent	present	suppressed

0 = not reported.

*Suppression test performed with 9 alpha fluoro hydrocortisone acetate (Florinef) 1.0 mg per day for 4 days. Results reported showed a decrease in 17 ketogenic steriods and a slight increase in 17 ketosteroids in the 24-hour urine specimen.

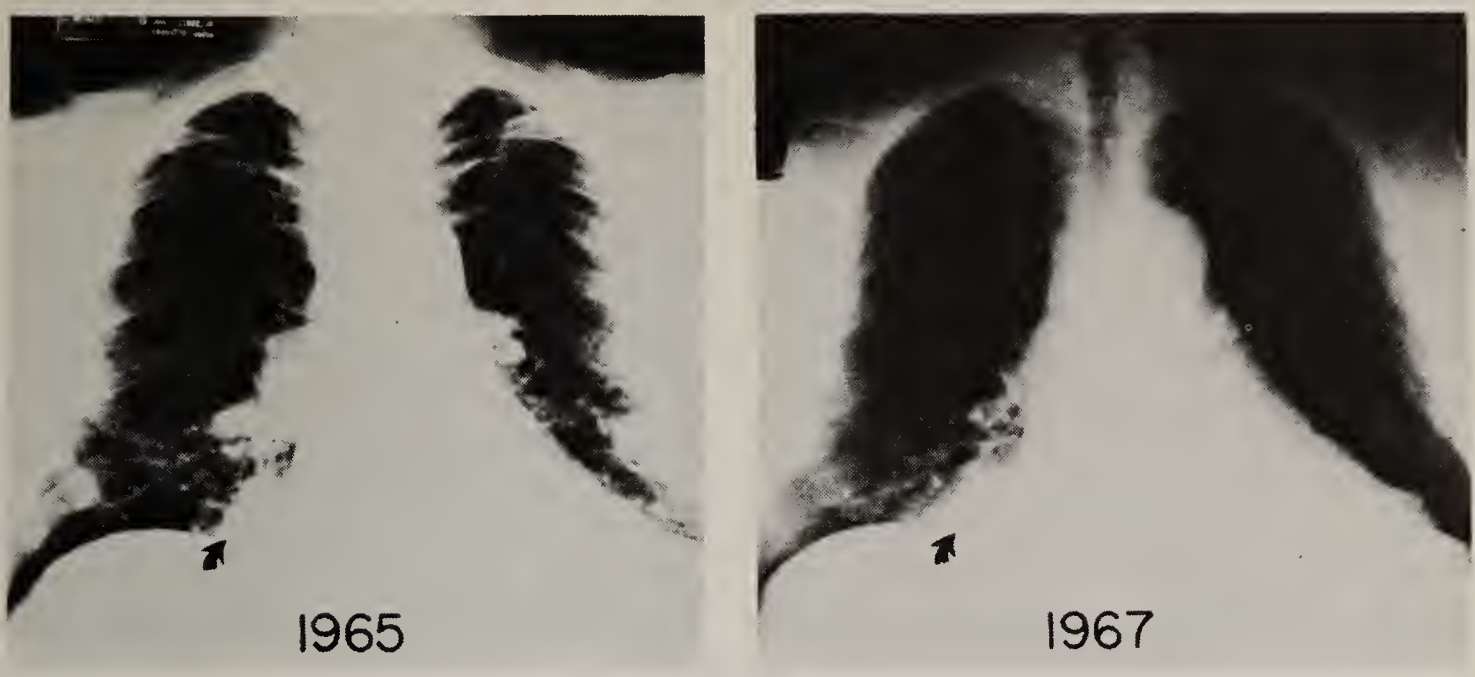

Fig. 1—Arrows indicate the abnormal density in the right cardiophrenic angle in the roentgenograms of the chest, obtained in 1965 and in 1967, of this patient.

Fig. 2—Laminogram obtained in 1967 of the density in the right cardiophrenic angle.

brospinal fluid of this patient was first noted in 1965 and confirmed during his admission in 1967. Analyses of cerebrospinal fluid in patients with ACTH-producing tumors have not previously been reported. However Trethowan and Cobb[13] examined the cerebrospinal fluid in nine patients with Cushing's syndrome and found elevated protein (51 to 144 mg/100 ml) in six patients. Furthermore they report that, following removal of adrenal cortical adenomas from two patients, the protein concentration in the cerebrospinal fluid markedly decreased within three weeks (in Case 2, a decrease from 124 to 40 mg/100ml, and in Case 24, a decrease from 144 to 46 mg/100ml. One might speculate that if the alteration of capillary permeability so frequently observed in patients with Cushing's syndrome also involved the capillaries of the central nervous system, then elevation in cerebral spinal fluid protein could be expected. Certainly, cerebrospinal fluid analysis in additional patients with Cushing's syndrome is warranted.

Hypokalemia is reported by different investigators to be both frequently[14] and rarely[15] associated with Cushing's syndrome. Correlation between the severity of the electrolyte disturbance and the concentration of plasma cortisol has been described.[14,16] The available information on serum electrolyte concentrations reported in 18 patients with Cushing's syndrome and bronchial adenomas is summarized in Table 2. Only four patients were reported to have normal serum potassium concentrations. Although the etiology of this electrolyte imbalance is not entirely clear, most investigators agree that hyperaldosteronism is unlikely since the concentration of this steroid is found to be normal or slightly decreased in most of the patients reported.[14,16] Regardless of the etiology

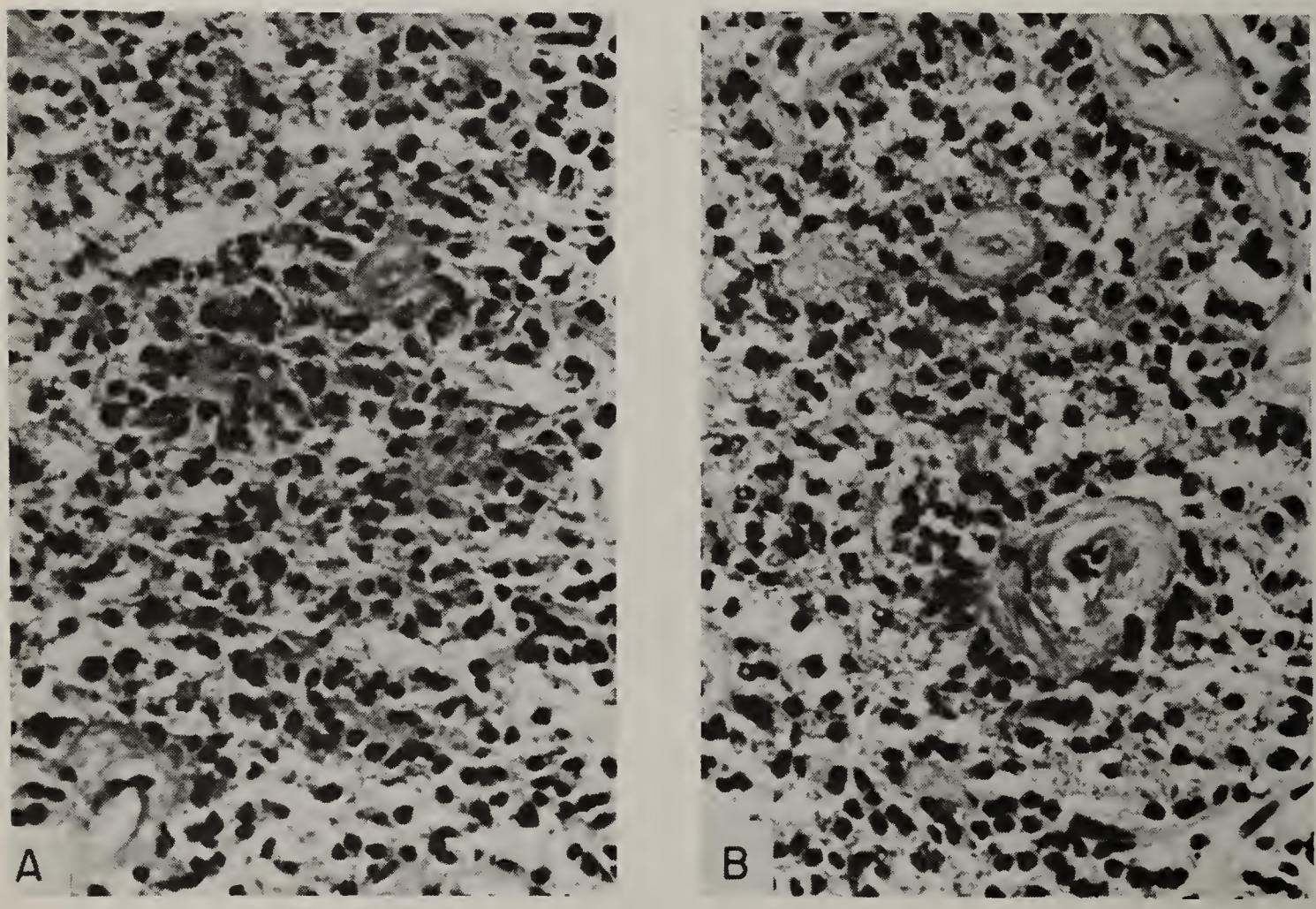

Fig. 3—Photomicrographs of the neoplasm surgically removed from this patient, magnified 480 times. Part A represents the carcinoid and Part B represents the cylindroid areas of this neoplasm.

of this abnormality, hypokalemia should be considered only a diagnostic bonus in the evaluation of the patient with suspected ACTH-producing adenoma; its absence should not be construed as strong evidence against the diagnosis.

To evaluate the pituitary-adrenocortical interaction in our patient, the dexamethasone suppression test, as described by Liddle[6] was performed. Suppression of elevated urinary 17-hydroxysteroids by 50%, after ingestion of 2 mg of dexamethasone, in patients with adrenocortical hyperplasia is expected.[6] Patients in whom the adrenal cortex is secreting autonomously, *i.e.*, patients with the ectopic ACTH syndrome and those with either an adrenocortical adenoma or carcinoma, usually do not show suppression in urinary 17-hydroxysteroids with the high dose of dexamethasone.

As can be seen by the data presented in Table 1, the concentrations of urinary 17-hydroxysteroids were decreased from 19.5 to 6.3 mg following ingestion of 8 mg of dexamethasone. This finding became less disconcerting when a review of the literature (Table 2) revealed that suppression of 17-hydroxysteroids with 8 mg of dexamethasone has been reported with increasing frequency in patients with Cushing's syndrome associated with bronchial adenomas (Table 2) and other neoplasms.[30, 31, 32] A possible explanation is simply that hormone production from ectopic sites or from the adrenal cortex[33] is not always autonomous. If these neoplastic cells contain information essential for hormone production, there is no reason to deny the possibility that they might, in addition, contain information for regulation of hormone release.

The ACTH stimulation test[34] is a measure of the ability of the adrenal cortex to increase its secretion of steroid hormones. This procedure was carried out in ten patients with Cushing's syndrome associated with a bronchial adenoma, summarized in Table 2. The quantity of endogenous ACTH present in the plasma apparently was not sufficient to produce maximal 17-hydroxysteroid secretion since administration of exogenous ACTH resulted in an additional increase in adrenocortical hormone release. The authors are indebted to Dr. Grant W. Liddle for performing the ACTH assays on the tumor* (10.3 m units/g) and post-surgical plasma (less than 1.5 m units/100 ml) specimens. Without this information the diagnosis of ectopic ACTH syndrome could not have been clearly substantiated.

SUMMARY

A patient with Cushing's syndrome secondary to an ACTH-producing adenoma of mixed carcinoid and cylindroid type is presented. Suppression of 24-hour urinary 17-hydroxysteroids (19.5 to 6.3 mg) and 17-ketosteroids (20.6 to 7.5 mg) followed oral administration of 8 mg of dexamethasone. Stimulation of the adrenal cortex with exogenous ACTH resulted in additional secretion of steroid hormones. A review of the laboratory data on our patient and 18 other similar patients described in the literature is presented and discussed. Spironolactone-induced false elevation of plasma cortisol as determined by the fluorimetric assay is demonstrated.

*Dr. Liddle informed us that 10.3 m units/g of tumor is 15th from highest concentration of ACTH found in a series of 83 tumors assayed for ACTH in his laboratory.

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A POST-DOCTORAL HOSPITAL-BASED COURSE IN MEDICAL ENGINEERING

Robert C. Arzbaecher

Medical researchers and engineers are working together in an increasing number of areas. For the physician, this collaboration has yielded new methods of analysis and sophisticated experimental instruments. For the engineer, medical research presents problems challenging because of their size and interdependence, because of the non-linearity and adaptability of the physiological systems involved, and because of the high degree of social value and personal interest attached to their solution.

A reasonable amount of general training in human physiology will be very helpful to the engineer involved in such collaborative activity, and biomedical engineering programs now exist all over the country to provide such training. On the other hand, it is also quite possible to provide the medical scientist with engineering lore sufficient to enhance his potential for a fruitful liaison with an engineer. This paper describes such an effort.

The Department of Biomedical Engineering at this institution conducted an intensive eight-week course in biomedical engineering during July and August, 1969. The course was open to members of the attending staff, house staff, and fellows. Four subject areas were covered: engineering mathematics, analog computation, digital computation, and electronics.

Notice of the program, course descriptions, and a timetable were sent to al members of the medical and scientific staffs. Although registrants were encouraged to commit themselves full time to the entire program, allowance was made for those who were able to enroll for only one or two courses. Full-time students were given study and work space in the Department of Biomedical Engineering. Some were residents who arranged with their divisional chairmen a formal two-month rotation through the department. Others used part of their research year or vacation. The faculty* consisted of four biomedical engineers (members of the Scientific Staff), two assistant attending surgeons, and an engineering graduate student who designed the laboratory experiments and assisted in electronics. In addition, four of the department's thirty graduate students currently working toward a Ph.D. in bioengineering were assigned as special tutors.

From the Department of Biomedical Engineering, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Robert C. Arzbaecher, Ph.D., Associate Attending Biomedical Engineer

*James W. Dow, M.D., Coordinator, Robert C. Arzbaecher, Ph.D., Peter Biancalana, B.S., Jerold S. Brodkey, M.D., John A. Campbell, Ph.D., David L. Roseman, M.D., William D. O'Neill, Ph.D., and Allen A. Sandberg, Sc.D.

FACILITIES

Many of the lecture and laboratory sessions on electronics and analog computers were held at the science and engineering laboratories of the University of Illinois Chicago Circle Campus, one mile from Presbyterian-St. Luke's Hospital. There was no difficulty in arranging to use the University's complement of analog computers and electronic equipment, and the students' laboratory work was arranged on a two-to-a-station basis.

The lectures in engineering mathematics and the digital computation course were located in the Department of Biomedical Engineering research area at Presbyterian-St. Luke's Hospital. Here the class had "hands-on" access to a well-equipped scientific computer center. In addition to its routine computational function, this center has developed specific programs and interface equipment for extensive signal processing procedures. Students had an unusual opportunity to become familiar with on-line, real time acquisition and analysis of biological data.

Table I shows the class schedule. On Monday and Friday the students spent the mornings in electronics, and the afternoons in analog computation. Classes began with a lecture-discussion period which then led directly to the experiment for the day. On Tuesday and Thursday mornings there was a lecture-work session in digital computation. Wednesday morning's lecture concerned the theoretical aspects of electronic systems, and the computer laboratory was then opened for individual projects. The afternoons of Tuesday, Wednesday, and Thursday featured a lecture in engineering mathematics, while the remainder of the days were free for study and homework.

Table II lists the instruments used in the program. Routine measuring equipment—power supplies, amplifiers, oscillators, oscilloscopes—were shared by two students. The more sophisticated tape recorder and signal averager were first explained and demonstrated to the group as a whole. Later these instruments were available for private detailed familiarization and experimentation. There were five anolog computers altogether, plus a plug-in patchboard for each student. In addition, an analog computer was made available for after-hours use in the students' study area. The digital computer was "reserved" during the scheduled sessions and also was frequently available at other times during the day and night.

CURRICULUM

The course was intended to provide the research-oriented physician with a new set of tools which would be helpful to him in the conduct of modern medical research. But, more importantly, we hoped to instill a feeling for those engineering concepts which are being increasingly applied to medical problems: system identification, measurement and analysis, simulation, statistical methods, signal processing and mathematical modeling, and the use of digital computers in data reduction and calculation. The material selected for presentation and the laboratory exercises were only a sampling of possible areas of study. Both were covered in sufficient depth to allow detailed mathematical treatment and, in the case of electronic devices, considerable physical insight. To a large extent the engineering applications toward the end of the course were those involving physiological systems. Early work was basic and devoted to systems much simpler and more well-behaved than biological ones.

Table III details the lesson-plan for all four courses in such a way as to place in evidence the mutual dependence of the courses and their final merger into a unified treatment of signal and system theory. The engineering mathematics course began with some notions about functions and graphs; proceeded through slope and area, differential and integral calculus; and ended with differential equations. The analog computer course

TABLE I. CLASS SCHEDULE

Hours	Monday	Tuesday	Wednesday	Thursday	Friday
9-10	Elec Lect	Dig Comp Lect	Elec Lect	Dig Comp Lect	Elec Lect
10-12	Elec Lab	Dig Comp Lab	Dig Comp Lab	Dig Comp Lab	Elec Lab
12-1	Free	Free	Free	Free	Free
1-2	Ana Comp Lect	Math	Math	Math	Ana Comp Lect
2-4	Ana Comp Lab	study/work	study/work	study/work	Ana Comp Lab

NOTES:

Math—Lectures in engineering mathematics including a selected introduction to function theory. Differential and integral calculus, and differential equations.

Dig Comp—Lectures and laboratory practice in the theory and operation of digital computers, including instruction in Fortran programming, numerical techniques, analog to digital conversion and processing of physiologic signals.

Ana Comp—Lectures and laboratory practice in the theory of analog simulation, operation of electronic analog computers and linear system and signal theory applied to models of living systems.

Elec—Lectures and laboratory practice in the theory of electronic circuits, operating characteristics of medical research instruments, sensitivity, noise, bandwidth and transducer problems in medical electronics.

TABLE II
INSTRUMENTS USED BY STUDENTS IN LABORATORY WORK

Dig Comp	IBM 1800 computer, including 24 K memory, 2 tape drives, triple disc pack, line printer, digital plotter, analog to digital (16 channels) and digital to analog (8 channels) converters, teletype remote terminals
Ana Comp	EAI TR20 analog computers
Electronics	Vacuum tube and transistor power supplies, audio sinusoidal oscillators, low frequency function generators, 2-channel general purpose oscilloscopes, x-y recorders, 2-channel strip-chart recorders. 7-track magnetic tape recorder, storage oscilloscope, signal averager, potentiometric recorders

gave physical reality to the mathematical concepts and immersed the student in signal processing procedures such as summation, integration, differentiation, and function generation. The input-output relationships—"transfer functions"—so essential to modern engineering were nicely observed in the analog computer laboratory. Simulations of the respiratory and cardiovascular systems were performed during the last two weeks of the course.

At the same time, facility in transduction, amplification, processing, and display of measured quantities was being developed in the electronics course. Diode and amplifier circuits using transistors were constructed and analyzed. Digital circuits such as the multivibrator and/or gate and flip-flop were examined in detail, and the students were introduced to logical design of simple computer networks. The last three weeks of the electronics course were spent using typical physiological instrumentation, with emphasis on the effects of noise, frequency response, and sensitivity. Included were electrocardiographs, strain gage pressure transducers connected to indwelling arterial catheters, nuclear counters, scalers, scintillation detectors, and pulse height analyzers.

TABLE III

COURSE OUTLINE

Week	Engineering Mathematics	Analog Computation	Electronics	Digital Computer
1	Theory of functions. Real number systems.	Characteristics of operational amplifier and computer operation.	Basic electric circuit theory. Sinusoidal behavior of networks. Impedance models.	Hardware. Software.
2	Rates of change as ratios of differences.	Programming algebraic equations.	Frequency reponse. Filters. Operational amplifiers. Integrators. Analog circuits.	Software and hardware interaction.
3	Derivatives of functions. Difference ratios as approximate derivatives.	Differential equations.	Semiconductor fundamentals. Diodes and diode circuits. Transistor.	Fortran IV programming methods.
4	Sums as approximate areas. Integrals as limits of sums.	Magnitude and time scaling.	Transistor amplifier load lines. Quiescent operating point. Ideal amplifiers. Field effect transistors.	Input/output to computer. Printer, typewriter and plotter.
5	Approximate integration. Integral as an operator.	Multiplication and division of functions. Non-linear differential equations.	Logic gates. Counters. Logic circuits.	Scientific subroutine package. Random techniques.
6	Cascaded integral operators. Differential equations.	LaPlace transform. Transfer functions.	Biotransducers and electrodes. Recording of physiologic signals.	Data storage: disk, magnetic tape.
7	Differential equations and operators. LaPlace transforms.	Construction of models from physiologic input/output characteristics.	Step & sinusoidal response. Bode plots. Systems and stability of systems.	On-line use of 1800. Analog input/output. Remote communications.
8	Systems analysis.	Analog computer investigation of systems and stability.	Modern instruments for biochemistry and nuclear medicine.	Conversational languages. Conversational Programming System.
Texts	Frank Ayres, *Schaum Outline of College Calculus,* New York, McGraw Hill, 1968.	V. Joseph Blum, *Introduction to Analog Computation.* New York, Harcourt Brace & World, 1969.	James Brophy, *Basic Electronics for Scientists.* New York, McGraw Hill, 1966.	Robert Louden, *Programming for the IBM 1130 & 1800; IBM 1800 Manuals.*

DISCUSSION

Since its termination, we have had some opportunity to reflect on the course and to form some opinions for future offerings. To begin with, staff reaction to the proposed program was generally enthusiastic. However, many of those who wished to enroll were unable to do so because of the pressure of other responsibilities. These persons felt that nighttime or late afternoon scheduling of one course at a time would better fit most needs.

On the other hand, it was generally agreed that the four courses complemented each other and that a full-time commitment to the whole program would produce a deeper awareness of the engineering concepts and a more integrated approach to the subject. Eight staff members registered for the full program, and so we went forward with our plans. Another four people participated on a single-course basis.

The small enrollment assured adequate laboratory experience, and this was perhaps the strongest point in the program's favor. Particularly important was the opportunity for "hands-on" contact with the electronic instruments and computer consoles. Even so, the four courses could have contained twice their actual enrollment without straining our facilities; in our future planning we hope to utilize our resources better by raising the student-faculty ratio from 2:1 to 4:1. The auxiliary tutorial system will then be used to better advantage.

Further thought should be given to an evening program, one course at a time. The demand for such is clear.

A key development in the course was the introduction, in the last two weeks, of some ideas in mathematical modeling of a physiological system. Here the class was encouraged to lead the way. We used a respiratory example involving CO_2 concentration during rebreathing, though many equally suitable examples are available. The class contributed some "data" which were judged by all to be reasonable or representative. The class then suggested a tentative model, based on known anatomical or physiological structures (e.g., a compliant compartment having a fixed leakage rate and, perhaps, orifice resistor.) The response of the model to a sudden change in input was calculated from its mathematical structure and compared with the known data. The necessity for some gross changes in the model became obvious from a consideration of final steady-state conditions, and these resulted in a new configuration which was still physiologically appealing. Gradually, the response of the model became morphologically similar to the data, and then numbers emerged.

Teaching modern engineering courses to people in academic medicine is a rewarding challenge to both teacher and student. The kind of teacher-student exchange described in the last paragraph occurs naturally during an attempt at mathematical representation of a physiological system; it should be the final goal of any course of this nature and the initial stepping stone to much more fruitful interactions for both parties.

RUSH PRESBYTERIAN-ST. LUKE'S

MEDICAL BULLETIN

VOL. 9 NO. 3 JULY 1970

RENAL AND NUTRITION SYMPOSIUM

Hunger and Malnutrition in the United States

Recent Advances in Peritoneal Dialysis

Renal Synthesis of Ammonia and its Implications

Prostaglandins in Health and Disease

Recurrent Infection of the Urinary Tract: Renal and Bladder Bacteriuria

Body Composition in Chronic Renal Failure; Effects of Diet

RUSH PRESBYTERIAN-ST. LUKE'S MEDICAL BULLE

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Editor's Note

On October 24, 1969 Presbyterian-St. Luke's Hospital signed an agreement of merger with the Trustees of Rush Medical College forming a new corporation known as Rush Presbyterian-St. Luke's Medical Center. The new medical center was created to reactivate the Rush charter and re-establish the undergraduate educational programs of Rush Medical College. The time has now come when the "Bulletin" must change its title to conform with the new academic setting. There are no present plans to change the nature of the publication, and the scope of its distribution will be the same as before.

The new title of the publication will be *Rush Presbyterian-St. Luke's Medical Bulletin*. And thus there may be some changes in alphabetical filing as far as libraries are concerned.

The present issue is distinguished by articles presented at a symposium inaugurating a Section of Renal Diseases and Nutrition. Dr. Robert M. Kark, director of the section, has written a foreword to the papers that are presented. The *Bulletin* is pleased to carry the reports of this symposium, some of which may, because of lack of space, appear in a subsequent issue.

Evan M. Barton

FOREWORD

Up-to-date facilities in a medical school provide a better environment for learning than cramped and grubby ones. It is true that some Nobel Prize winners have made their great discoveries in a filthy basement using a ball of string and some wax, but we are only incidently concerned with them. Our concerns are medical students, house officers, fellows and their education. The new facilities for the renal and nutrition section were developed with the above in mind. The idea was ten years old and the cost effectiveness had been worked over many times before a committee was formed to collect funds for it. The new facility contains the Women's Board Library and Seminar Room, offices, and laboratories, which occupy the 5th floor of the Rawson Building.

When the facility was completed, a small dinner was arranged to thank those who gave gifts to build the laboratory and those who worked hard to collect the gifts. In addition, a symposium on current renal and nutritional topics was planned with the generous help of friends in pharmaceutical companies. Some of the papers presented at this symposium make up the contents of this issue of the Rush Presbyterian-St. Luke's Medical Bulletin.

ROBERT M. KARK
Guest Editor

Robert M. Kark, F.R.C.P., Director of the Section of Renal Diseases and Nutrition, Department of Medicine, and Attending Physician on the staff of Presbyterian-St. Luke's Hospital, Chicago, Illinois

ACKNOWLEDGMENTS

The Section of Renal Diseases and Nutrition of the Division of Medicine of Rush Presbyterian-St. Luke's Medical Center officially inaugurated its new research, educational, and patient care facilities in April, 1970. The building program for the Section was completed in December, 1969. The new facilities include nutritional and metabolic laboratory, a physiology suite with associated isotope laboratories, rooms for immunology, chromotography, electromicroscopy and experimental histology, a unique set of laboratories for ultramicrochemical studies on isolated cells and fluids of the body, library and seminar room.

Funds for the building program were collected by a Committee composed of the following:

Herbert Bain	*American Meat Institute*
John P. Bent	*Rush Presbyterian-St. Luke's Medical Center*
Kenneth Carter	*Syntex, Mexico*
Albert B. Dick, III	*Rush Presbyterian-St. Luke's Medical Center*
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We would like to thank the following for support of the Symposium:

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The Joseph A. Davis Memorial Fund	Presbyterian-St. Luke's Hospital

PROBLEMS OF HUNGER AND MALNUTRITION IN THE UNITED STATES

James P. Carter

The work presented in this paper can be considered as a forerunner to the Tennessee area program in nutrition. This program is a collaborative effort between Vanderbilt University, Meharry Medical College, St. Jude Children's Research Hospital, and the Tennessee Medical Association, to study and implement demonstration programs to solve some of the problems of hunger and malnutrition not only in the State of Tennessee but elsewhere in the country. This program is being sponsored but not yet funded by the National Clearinghouse for Nutrition, of the Department of Health, Education and Welfare (HEW). The National Clearinghouse for Nutrition is also conducting the National Nutrition Survey. Some data will be presented from the National Nutrition Survey but much of these are not available for release, especially as they pertain to the particular states which have been surveyed.

BACKGROUND

Nearly three years ago, the Field Foundation, Incorporated sent a team of doctors into Mississippi. Their report on hunger shocked and embarrassed the nation and provoked testimony before congressional committees, much television coverage and other publicity. After this report, an *ad hoc* committee was established, called the Citizen's Board of Inquiry into Hunger and Malnutrition in the United States. This Committee, composed of a group of concerned private citizens, was financed by a consortium of private foundations. On a meager budget, it put together a report entitled *Hunger, U.S.A.* This document discusses hunger and the lack of response to need in Mississippi, as well as problems associated with the documentation of hunger and malnutrition in the United States generally, our present food and welfare programs, and agricultural policy. It makes specific recommendations as to what should be done to alleviate the crisis. In reviewing the document, *Hunger, U.S.A.*, in preparation for this presentation, I could not help but be struck with how much we learned and how extensively we covered the problem at that time. Also I could not help but see how little has been done and how ineffective present programs are in getting food into the hands of those who need it. In the words of Senator Walter Mondale, "we have flattered American's hungry with a mammoth amount of publicity; surveys; reports; T.V. documentaries; presidential messages; testimony; touring committees; and a White House Conference. We have flattered them with everything except food."

The *Hunger, U.S.A.* report was followed by congressional hearings and the establishment of the Senate Select Committee on Nutrition and Human Needs, under the chairmanship of Senator George McGovern. This Committee has performed well in hearing and publishing the testimony of medical experts, of the poor who suffer from hunger and malnutrition, of administrators of food programs, of other senators, *e.g.*, Fritz Hollings of South Carolina, and of representatives of state and local public health

agencies. Conspicuous by their absence and lack of involvement at this time were representatives of the organized medical community. I think one of the reasons for this is that most medical schools teach little or nothing about nutrition. Some professors have been so conditioned by the occurrence of pellagra in the 1930's, that unless an individual has diarrhea, dermatitis, and dementia, he cannot possibly be deemed to have a nutritional deficiency.

The McGovern hearings gave impetus to the National Nutrition Survey which is being conducted under the direction of Drs. Arnold Schaeffer and Ogden Johnson. This survey documents without any question the existence of widespread malnutrition among low income groups in this country.

President Nixon then called his White House Conference on Food, Nutrition and Health, December 1-4, 1969. This Conference has been defined by its chairman, Dr. Jean Mayer of Harvard University, "an exercise in adult education." I think a more appropriate term would be "an exercise in futility." Speaking as the chairman of one of the panels having to do with methods of surveillance and monitoring of the nutritional status of the American people, I can say that some of the things that we recommended not to do, the Administration did anyway. I am referring specifically to their $2,000,000-a-year allocation from an already meager budget to determine the nutritional status of the "average" American. Such a survey will not feed anybody. As a matter of fact, it will tend to cover up our problems because the overall average, *i.e.*, a national summary nutrition-statistic, will tend to look pretty good.

Some Facts Concerning Hunger and Malnutrition in the United States. It is only fitting that we should begin with the first Americans, the American Indians, who are indisputably the last when it comes to nutritional status. Figs. 1 and 2 show some of the children with severe protein and calorie malnutrition, *i.e.*, kwashiorkor and marasmus, studied on the Navaho Indian Reservation. The United States Public Health Service Indian Hospital in Tuba City, Arizona, serves only a portion of the Reservation. Yet there were 44 cases of kwashiorkor and marasmus over a five-year period. The age distribution of the cases is shown in Fig. 3. Most of the cases of marasmus were under 8 months of age. The cases of kwashiorkor ranged from 5 to 30 months of age, the majority of them occurring after one year of age. Forty-eight percent of the children with marasmus died and 13% of the children with kwashiorkor

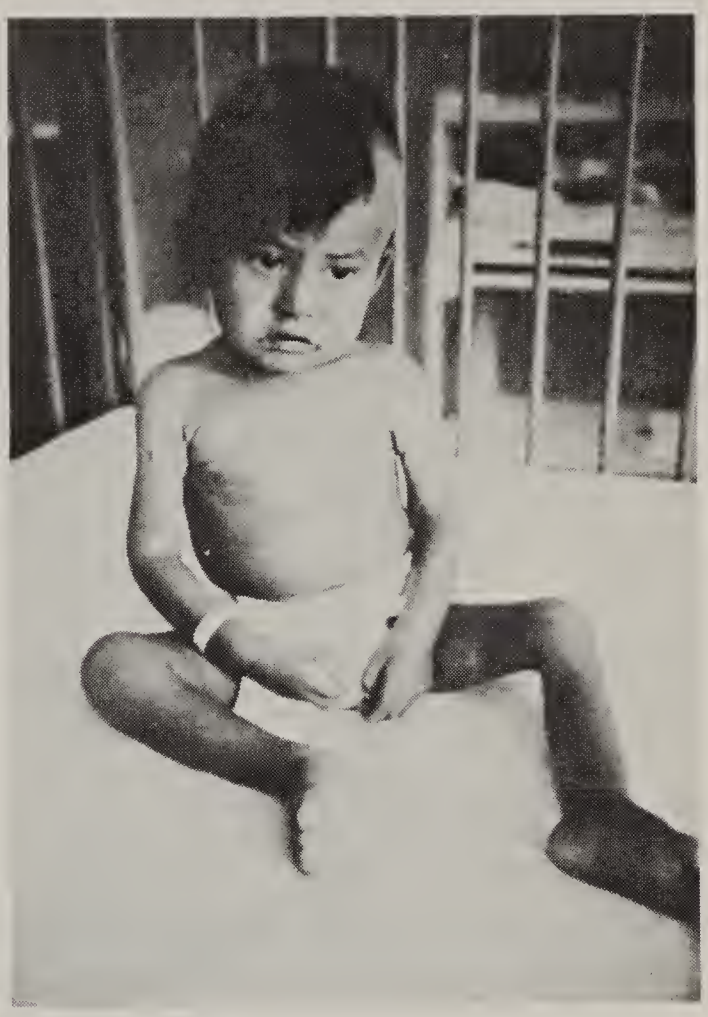


Fig. 1.—Navaho Indian child with malnutrition (see text).

died. The laboratory values are shown in Fig. 4. Note that serum albumin was less than 3.5 grams per 100 ml in all the patients with kwashiorkor.

There are also milder degrees of protein and calorie malnutrition as evidenced by growth retardation among the Head Start children, and this is widespread throughout the Reservation. With the help of school teachers, nearly 1,000 four- and five-year-old children were measured. An analysis of their heights is

Fig. 2.—Malnourished Navaho Indian children (see text).

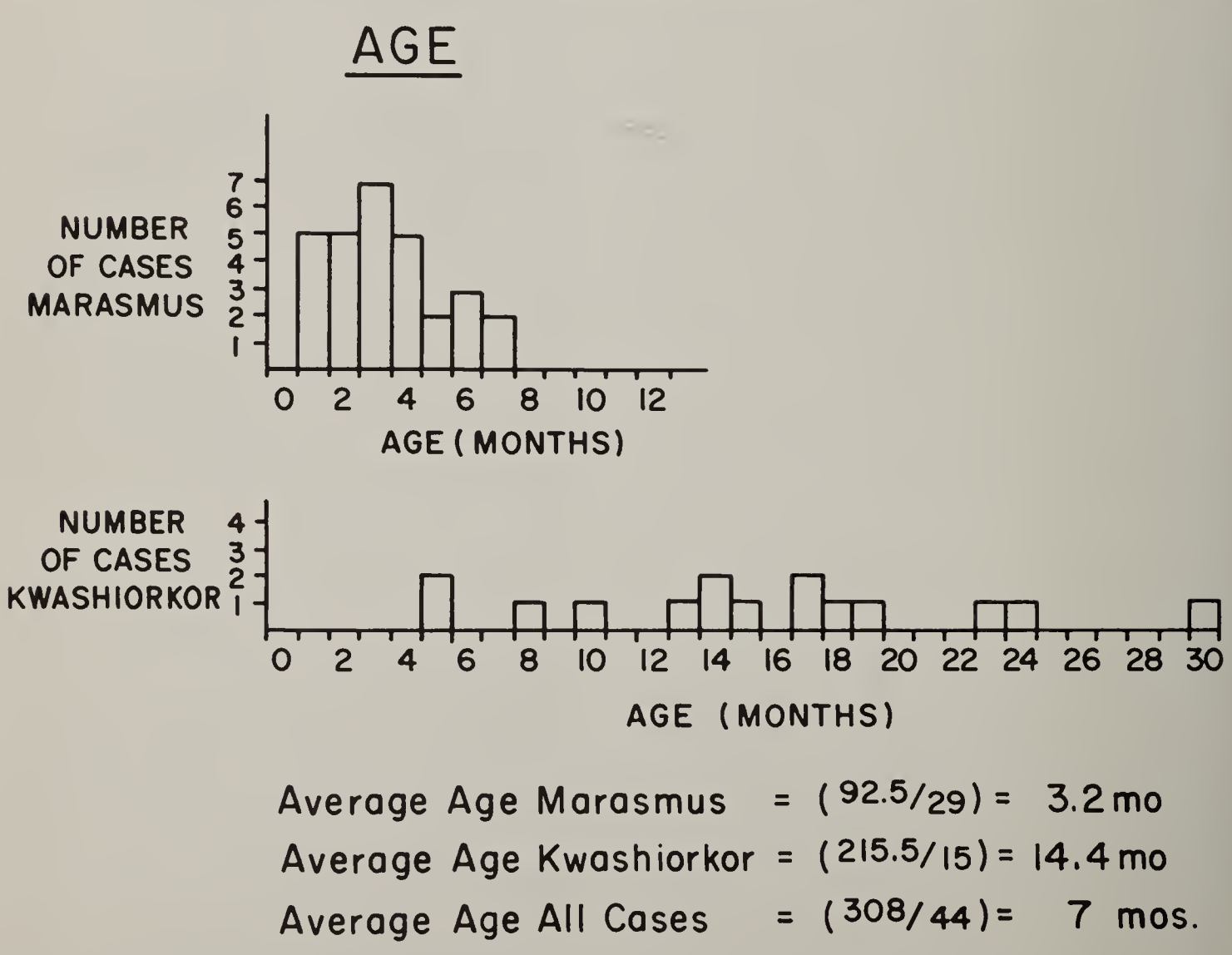


Fig. 3.—Age distribution of Navaho Indian children with marasmus and kwashiorkor.

given in Fig. 5. Nearly one-third of all of the Head Start children were below the third percentile for height on the Iowa growth curves.

If one examines the feeding histories of the children with kwashiorkor and marasmus, one finds that the majority of these children were not breast fed. In addition, infection is often associated with these syndromes. The most frequently associated infection was gastroenteritis, although gastroenteritis plus a respiratory infection was also often seen. It would appear that the syndrome of kwashiorkor and marasmus on the Navaho Indian Reservation, and probably on many other Indian reservations in this country, is no different from what it is in many developing countries throughout the world.

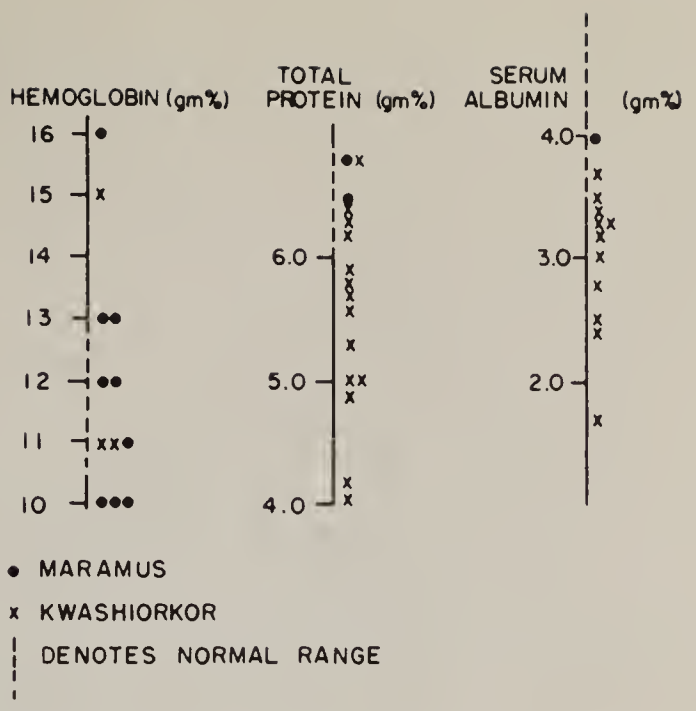
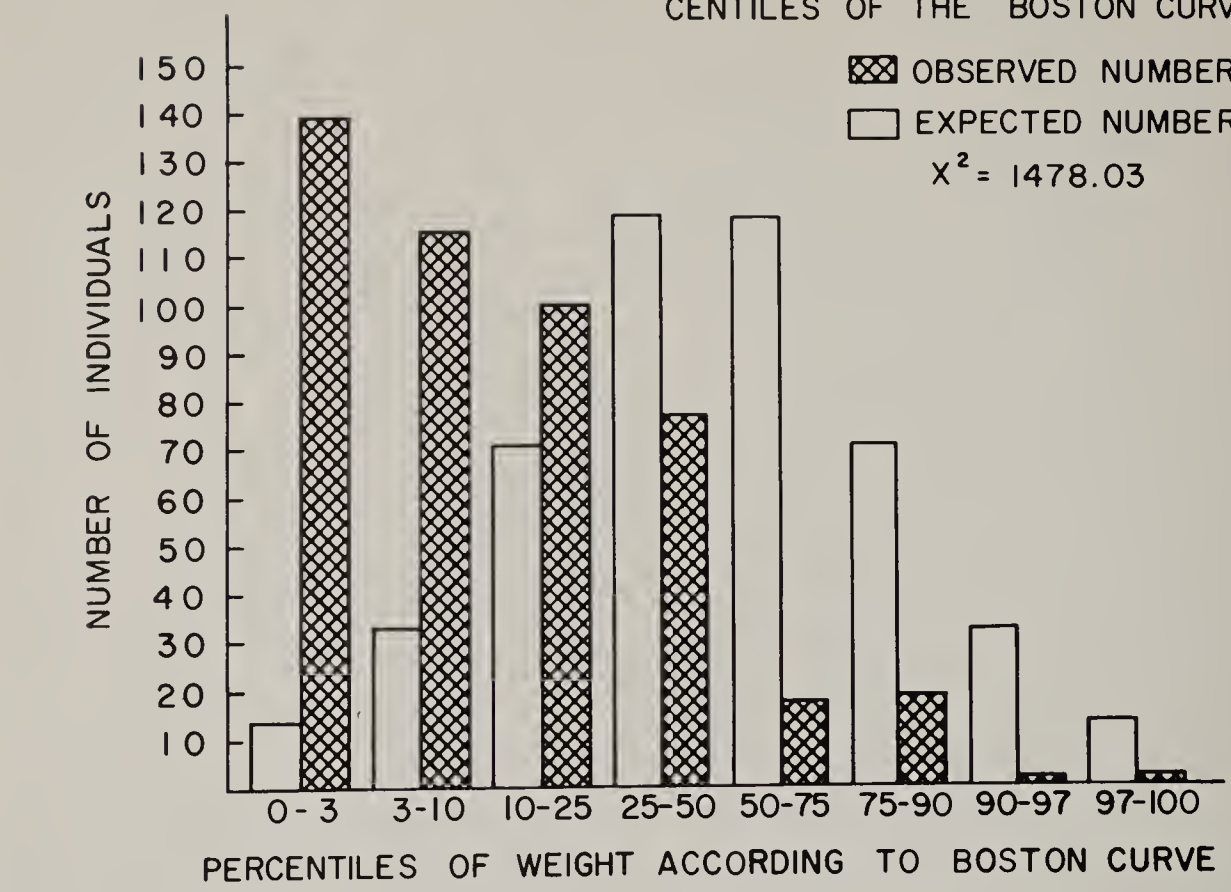


Fig. 4.—Laboratory determinations in Navaho children with marasmus and kwashiorkor.

In Nashville we first tried to obtain some information on the nutritional status of low-income groups by helping to design a questionnaire which was administered by the Center for Community Studies at George Peabody College for Teachers. The survey was conducted in 1968 on 594 families involving 2,057 individuals who were living in a high-poverty area of North Nashville and Southeast Nashville. The sample was selected to represent an estimated population of 64,498 people based on the 1960 census. Fifty-five percent of the family units were spending less than $5 per person per week for food (Table 1). According to the United States Department of Agriculture's (U.D.S.A.) own figures, this amount of money is insufficient for a family of four to purchase their Economy Food Plan which is considered an emergency measure. The Economy Plan will cost a family of four $100 per month or $6.25 per person per week. A better diet would be that of the U.S.D.A.'s Low Cost Food Plan which would cost a family of four $125 per month. Of course the logical question that listeners usually raise is, "Well, maybe they are spending the money for other things." The U.S.D.A. has partially answered this. Their own data from the 1965 Household Food Consumption Survey show that the amount of money spent for soft drinks, alcohol, potato chips, and ice cream goes up directly in proportion to income (Table 2), and the amount of money spent for food away from home goes up directly with income (Table 3).

Another question included in the questionnaire was "Is there anyone in this household who has eaten clay, starch, or dirt recently?" Eighty-five percent replied No, 2% said they didn't know, and 13% replied Yes (Table 4). The number of responses in the affirmative probably would have been higher in some of our rural areas in the southeast.

In order to lend further scientific force to these data, let us consider the findings of the Vanderbilt Nutrition Group working in conjunction with the Bill Wilkerson Hearing and Speech Center. They conducted a nutrition survey in two Day Care Centers in the same high poverty areas in North and Southeast Nashville mentioned above. Ninety children were examined. Except for non-specific nutritional signs, they were normal on physical examination (Table 5). The average income per family was $3,840 per year. Each child had, on the average, three to four siblings. As will be shown later, the income level of these families is considerably above that of families studied



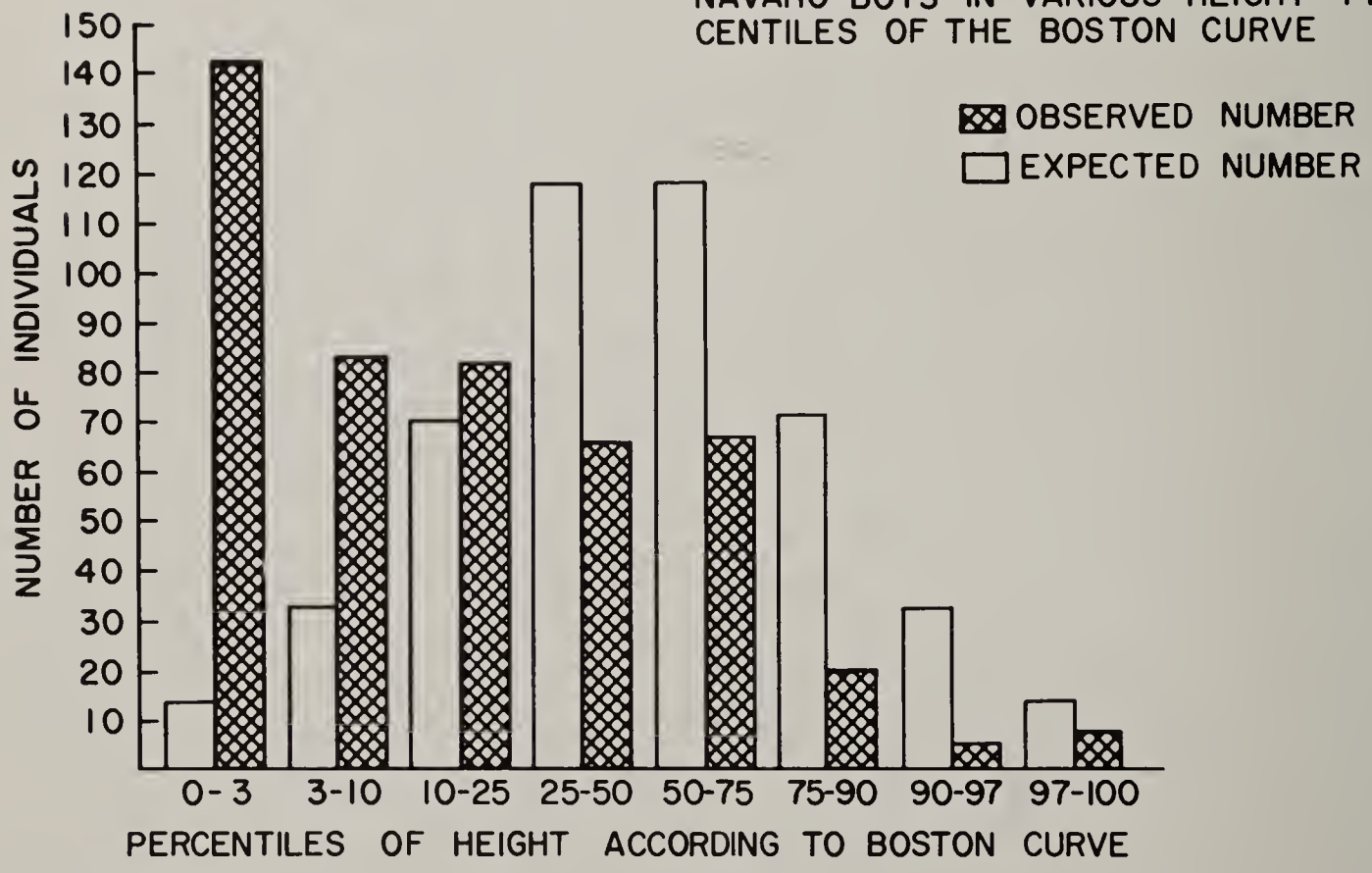


Fig. 5.

by us in South Carolina, and by Dr. Paul Zee and his associates, in Memphis, Tennessee.

The dietary data which we obtained on these families were qualitative only, because initially our dietitian was operating "Chicago-style" and going into the homes inspecting cupboards and refrig-

TABLE I

FOOD EXPENDITURES OF 594 FAMILIES IN HIGH POVERTY AREA OF NORTH NASHVILLE AND SOUTHEAST NASHVILLE DURING MARCH, APRIL, AND MAY OF 1968.

(The Family Health Study, Center of Community Studies, Peabody College).

Number of Individuals	Per Cent	Per Capita Expenditures For Food Per Week
394	19.2	less than $ 3.00
343	16.6	$ 3.00 to $ 4.00
399	19.4	$ 4.00 to $ 5.00
257	12.5	$ 5.00 to $ 6.00
159	7.7	$ 6.00 to $ 7.00
218	10.6	$ 7.00 to $ 9.00
149	7.2	$ 9.00 to $11.00
138	6.7	$11.00 and more

TABLE 2

This table shows money value spent by households in one week on various food and beverage items. These results from the 1965 National Food Consumption Survey show that spending on sweets, potato chips, soft drinks, and whiskey is related directly to income.

Income	Soft drinks	Alcoholic beverages	Potato chips	Ice cream
Under 1,000	$ .25	$ .11	$.04	$ .51
1,000-1,999	.26	.11	.02	.70
2,000-2,999	.43	.41	.09	1.04
3,000-3,999	.53	.42	.10	1.08
4,000-4,999	.57	.64	.14	1.33
5,000-5,999	.65	.98	.15	1.43
6,000-6,999	.72	1.18	.19	1.60
7,000-7,999	.79	1.49	.22	1.61
8,000-8,999	.74	1.43	.25	1.69
9,000-9,999	.75	1.60	.21	1.70
10,000-14,999	.87	2.12	.24	1.91
15,000 and over	1.16	3.79	.22	2.16

TABLE 3

DOLLARS SPENT WEEKLY BY FAMILIES FOR FOOD

Income	At Home	Away from Home	Total
Under $3,000	$15	$ 2	$17
$3,000-4,999	24	4	28
$5,000-6,999	30	6	36
$7,000-9,999	33	8	41
$10,000-14,999	38	13	51
$15,000 and over	46	19	65

erators. Cooperation in obtaining a recalled 24-hour dietary history was insufficient to produce quantitative information. Nevertheless, the data show that milk consumption was very low in seven children; five did not eat eggs; eleven consumed less than one ounce of meat per day; seventeen did not consume citrus fruits regularly, and six were using non-iodized salt. The growth achievement of these children was normal when compared with the Iowa growth standards (Figs. 6 and 7). Skinfold thickness determined with the Lange calipers was also within normal limits in these children (Fig. 8). Biochemically, however,

TABLE 4
ANSWERS TO QUESTIONS REGARDING KNOWLEDGE OF HOUSEHOLD MEMBER WHO HAS EATEN CLAY, STARCH OR EARTH

	Number	Percent
No	504	85
Didn't Know	12	2
Yes: (Reason Given)	76	13
Pregnant	26	—
Like It	28	—
Calms Nerves	3	—
Miscellaneous	9	—
Didn't Know	10	—
Total:	592	100

(The Family Health Study, 1968)

many of them had less-than-acceptable blood levels of one or more nutrients. Data on hemoglobin, mean corpuscular hemoglobin concentration, and serum iron are tabulated in Fig. 9. The hemoglobin levels were within normal limits. Twenty-seven percent of the children, however, had serum iron levels less than 70 micrograms per 100 ml. Serum folate levels were less than 4 millimicrograms per ml in 17% of the children. Erythrocyte transketolase activity and thiamine pyrophosphate effect were less than 750 mcg/ml/hr and greater than 15% respectively in nearly one-fifth of the children. Serum vitamin A levels were less than 20 mcg per 100 ml in 95% of the children; 11 of them had Vitamin A levels less than 10 mcg per 100 ml.

NUTRITION AND PARASITISM

Another question which has been of interest to us is the relationship between nutrition and parasitism. This has been studied in Beaufort County, South Carolina as part of a nutrition survey conducted jointly by Vanderbilt University, the University of South Carolina, and Meharry Medical College. The children examined lived in Bluffton and on Hilton

TABLE 5

SOCIAL AND ECONOMIC INFORMATION ON FAMILIES OF DISADVANTAGED CHILDREN
NASHVILLE, TENNESSEE 1968

	Number*	Average	Range
Income	81	$3840/yr	$1200-7200/yr
Siblings	85	3.4	0-14
Head of House	91	Father 58	
		Mother 29	
		Other 4	
Unemployed	4		

*The number of families reporting this information to the Social Worker.

Dietician's Social and Economic Observations on Sixteen Homes* (Twenty-nine children)

Food Costs $4.05/person/week (USDA estimate for Nashville = $5.63)
"Mother Hubbard Syndrome"—8 families

Employment and Education
Mother Employed —11
Mother Unmarried— 4
Mother Education —10 yr (avg)
Father Education —10 yr (avg)

Housing and Associated Items:

Slum	2	Range	15	Privy	2
Project	2	Refrigeration	15	Vermin	3 (1 bugs; 2 rats)
Old apt.	2	Electricity	15		
New apt.	4	Television	15		
"MC" House	4	Central Heat	13		

*Interviews attempted—38; achieved—16.

WEIGHT OF PRE SCHOOL CHILDREN FROM TWO NURSERIES
NASHVILLE-TENNESSEE 1968

Fig. 6.

HEIGHT OF PRE SCHOOL CHILDREN FROM TWO NURSERIES
NASHVILLE-TENNESSEE 1968

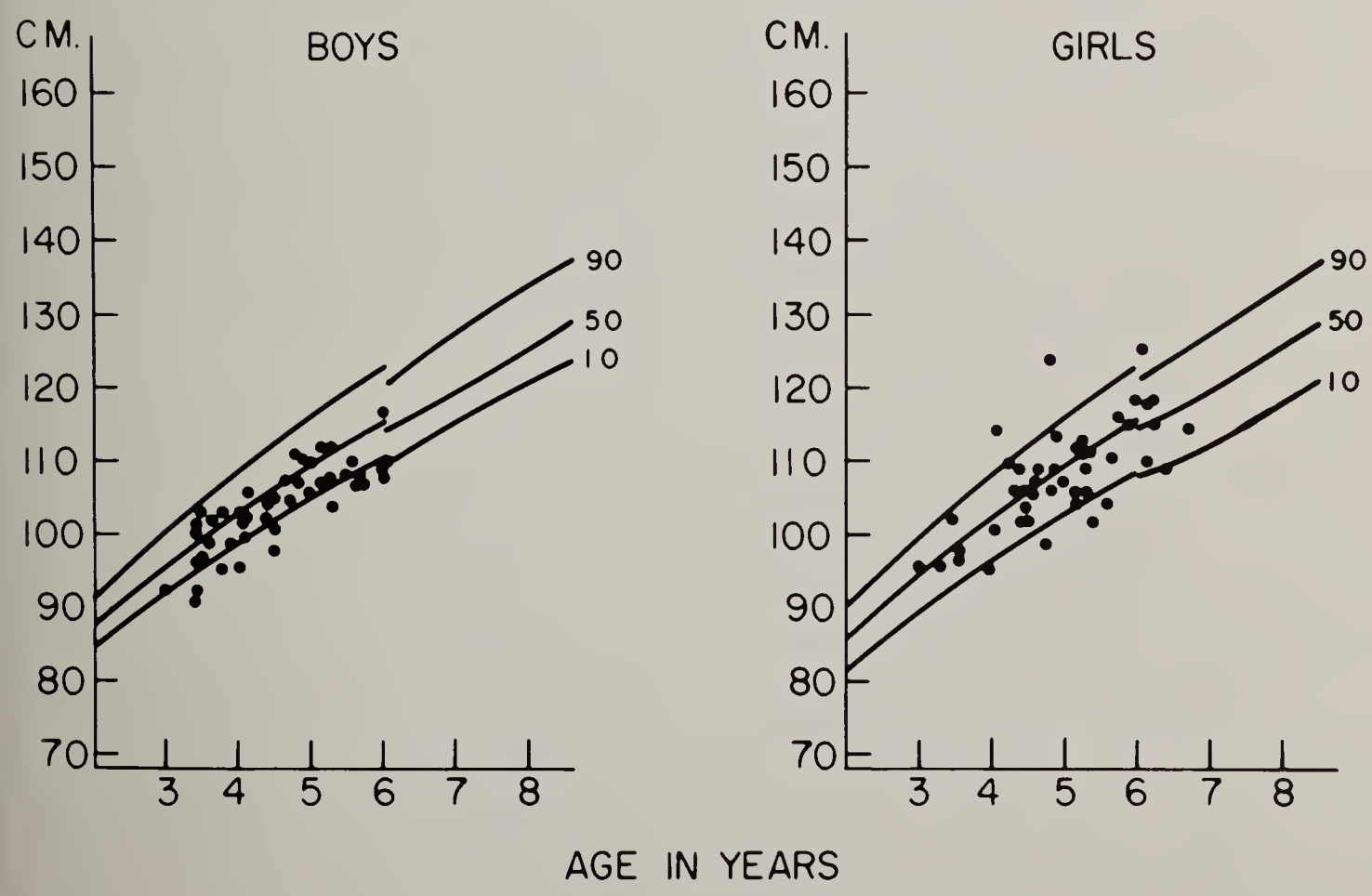


Fig. 7.

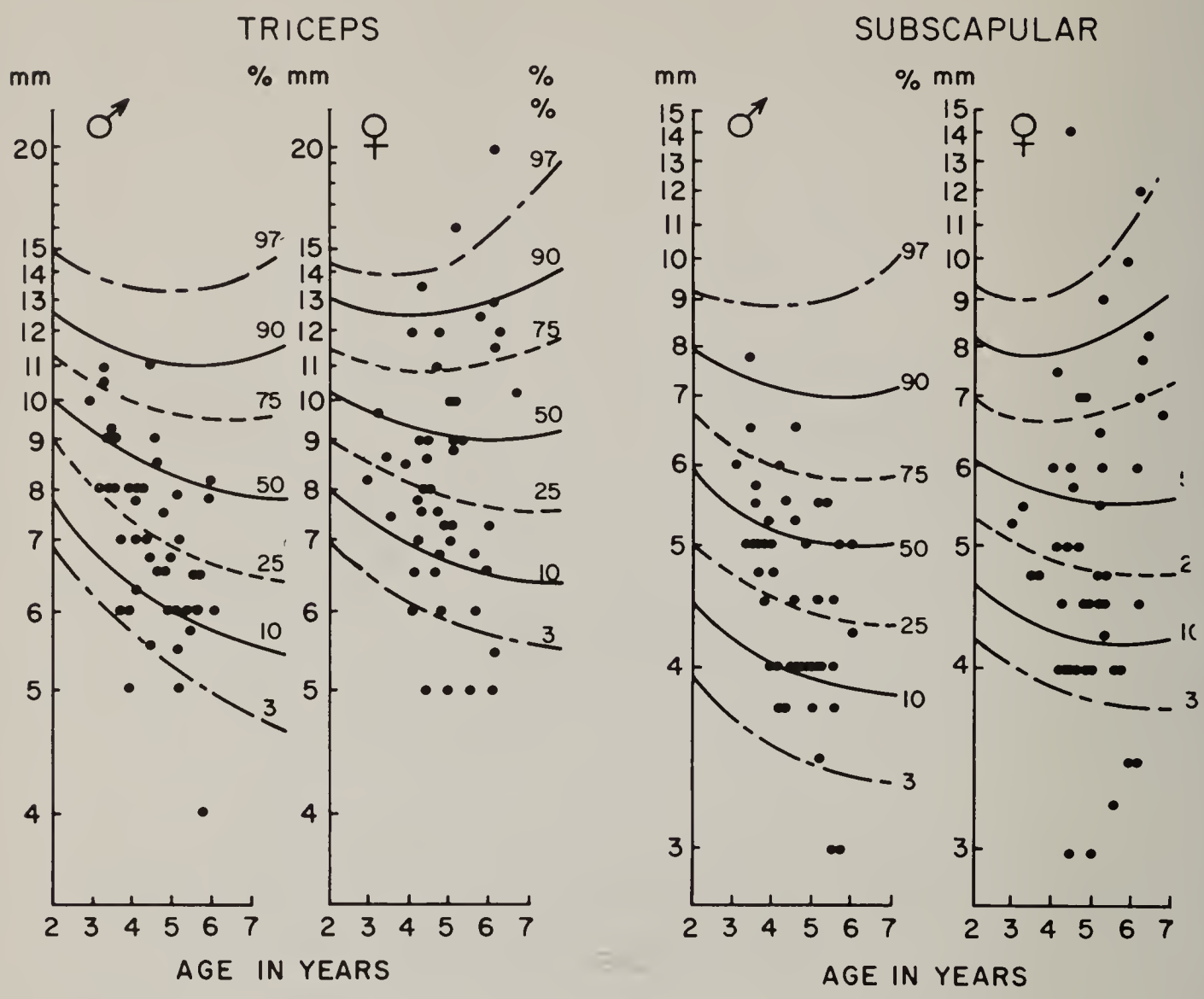


Fig. 8.

Head Island. The vast majority of families studied earned less than $3,000 a year. The type of housing, not at all unusual in many parts of the rural South today, lends itself to parasitism, since houses are raised above the ground (Fig. 10). If you collect samples of soil under the houses for analysis you frequently find the ova of *Ascaris lumbricoides*. Twenty-one percent of 131 black preschool children were infected with *Ascaris*; 25% were infected with *Trichuris*; 27% were infected with both. The overall prevalence rate of infection with either *Ascaris lumbricoides* or *Trichuris trichiura* for the black children was 73.3% (Table 6). We managed to examine a group of white children for comparison. Stool specimens were obtained from 46 children. Only one was infected with *Ascaris* and one was infected with *Trichuris*. The overall prevalence rate of infection for either of these parasites was 4.4% (Table 7).

The educational levels of the parents in both groups were the same. The differences were in economic status and the presence of indoor plumbing and toilets.

The average daily intakes of selected nutrients from 24-hour recall data—calories, iron, and vitamin A—are particularly low in the Negro children. The white children have higher intakes of every single nutrient with the exception of vitamin A. It is our feeling that the higher intake of vitamin A in the Negro children is due to the frequent consumption of greens.

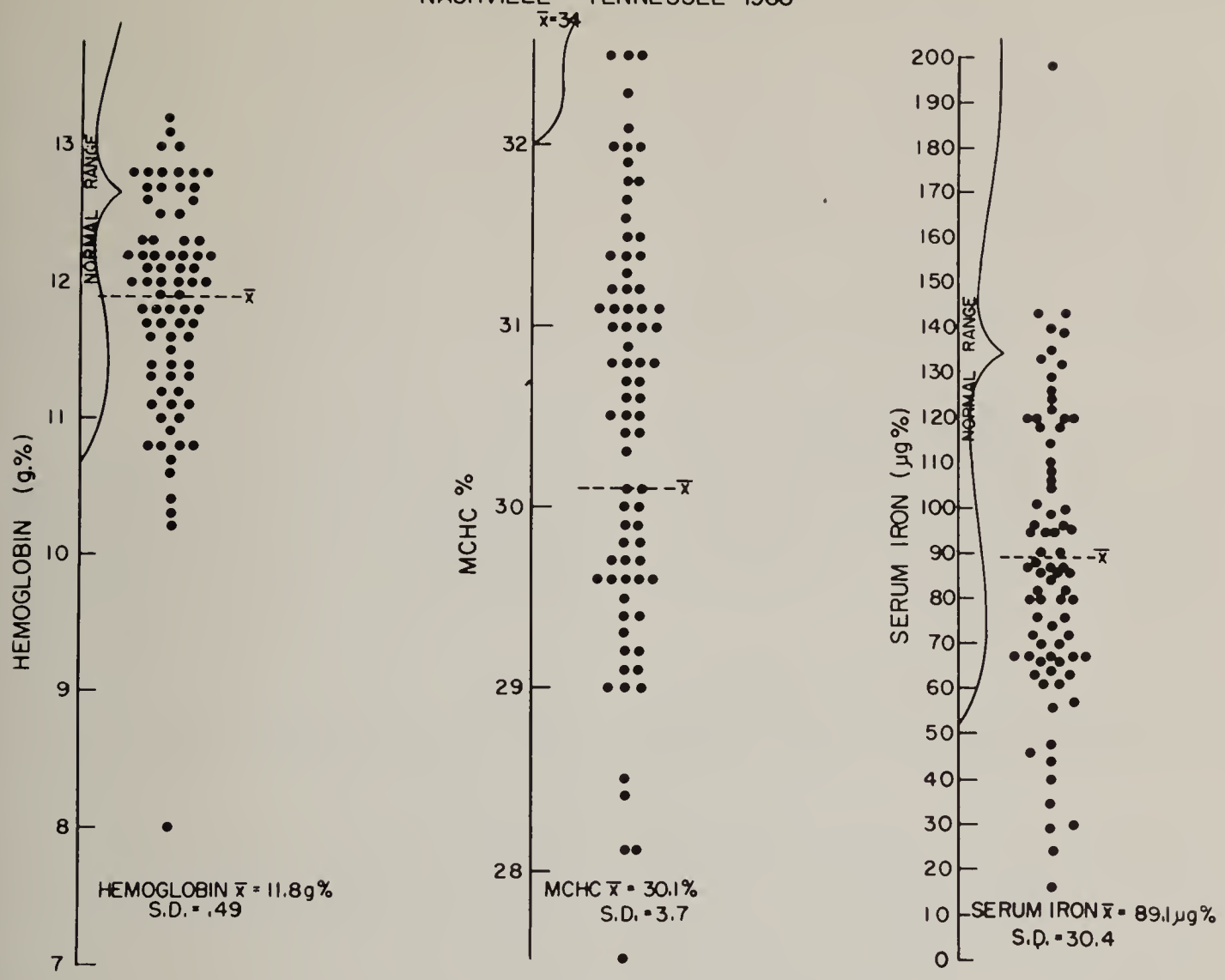


Fig. 9.

These children also suffer from growth retardation. There are greater numbers than expected in the lower percentiles for weight and for height (Figs. 11 and 12). The same is true for skinfold thickness as measured with the Lange calipers. There are greater numbers than expected in the lower percentiles, and the differences are highly significant. Measurements were taken in the mid-triceps area and in the subscapular area.

Ascaris lumbriocoides does not compete with the host for calories consumed. It has been estimated by von Brand that 50 adult ascarids would rob the host of less than one-half percent of a 2,500-calorie

TABLE 6

INTESTINAL PARASITES FOUND IN PRE-SCHOOL NEGRO CHILDREN
IN BLUFFTON-HILTON HEAD ISLAND AREA

	Number of Children	Per cent of Children
Infected with Ascaris only	28	21.4
Infected with Trichuris only	33	25.2
Infected with Both Ascaris and Trichuris	35	26.7
Not infected with Ascaris or Trichuris	35	26.7
Total Number of Stools Examined	131	100.00
Total Number of Children Infected with Parasites	96	73.3

TABLE 7

INTESTINAL PARASITES FOUND IN PRE-SCHOOL WHITE CHILDREN
BEAUFORT COUNTY, DECEMBER 1968

	Number of Children	Per cent of Total Examined
Infected with Ascaris only	1	2.2
Infected with Trichuris only	1	2.2
Infected with Both Ascaris and Trichuris	0	0
Not Infected with Ascaris nor Trichuris	44	95.6
Total Number of Stools Examined	46	100.0
Total Number of Children Infected with Parasites	2	4.4

diet or possibly one percent of a starvation diet. The worms, however, interfere with protein digestion and absorption. Also, *one* worm, if it migrates, can cause obstruction of the biliary tract and the tracheobronchial tree.

We did egg counts on a random sample of 30 infected children and estimated that each child had on the average between 10 and 50 adult worms. On the basis of the data of Venkatachalam and Patwardhan we estimated the protein loss for a load of 10 to 50 worms to be between ½ and 7 grams per day. This amount of protein loss is inconsequential with an average intake of 46 grams and certainly not enough to cause frank protein deficiency. We saw no cases of kwashiorkor, and the mean serum albumin was 4.5 grams per 100 ml.

On the other hand, *Trichuris trichiura* probably causes blood loss on the average of 0.005 cc per worm per day. This is based on the data of Dr. Layrisse and his group, from Venezuela. We estimated that those children harboring between 500 and 5,000 worms (one-quarter of those infected) lose between 2 and 25 cc of blood per day. These losses together with a low intake of iron in the diet could lead to severe iron deficiency and anemia. Fifty-one percent of the subjects examined had serum irons of less than 40 micrograms per 100 ml: 26% of the subjects had hemoglobins less than 10 grams per 100 ml. In contrast to the group of children that we examined in Nashville, only 30% of these children had serum vitamin A levels less than 20 micrograms per 100 ml.

In Memphis, Dr. Zee and his colleagues have been working with another population, and their preliminary data will be published shortly in the Journal of the American Medical Association. They have been working with black families who are refugees from rural Arkansas and the Mississippi Delta. Many of these children would be eligible for assistance from UNICEF or the Save the Children Federation if the government of the state of Tennessee or the United States Government requested it.

Fig. 10.—Typical rural southern house.

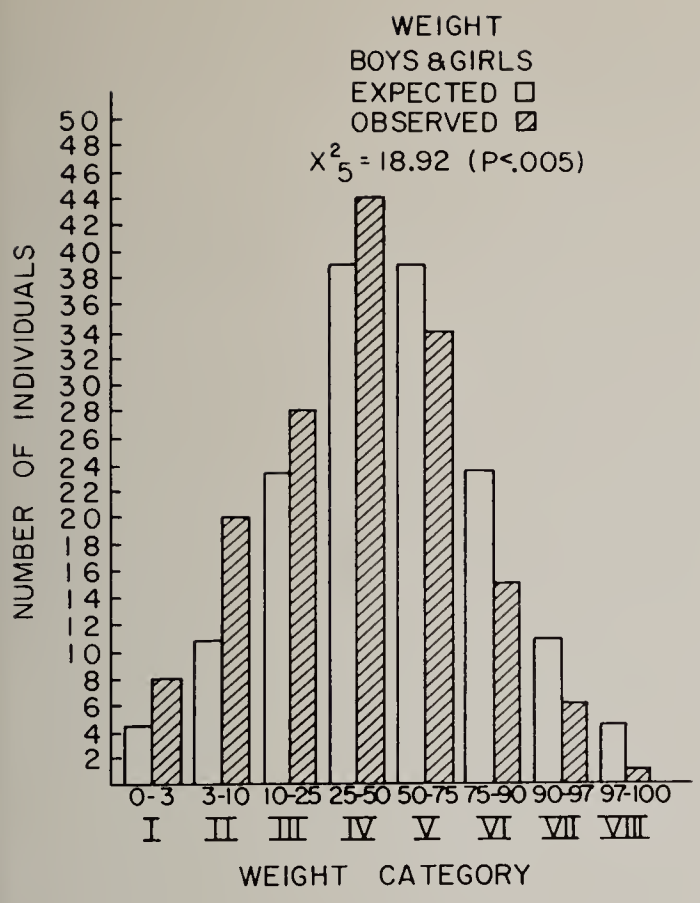


Fig. 11.—Weight of children studied in Bluffton and Hilton Head Island, South Carolina.

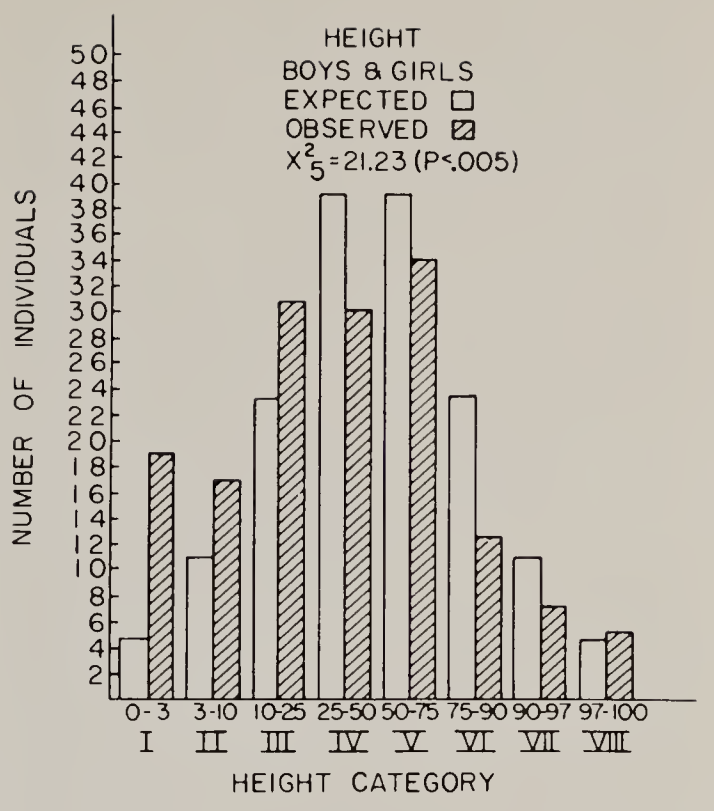


Fig. 12.—Height of children studied in Bluffton and Hilton Head Island, South Carolina.

Dr. Zee's group have sampled 300 children randomly selected from poor families living in south Memphis. The income level of these families is considerably lower than that of the families of the children that we studied in the Day Care centers in Nashville (Table 8).

The median annual family income was $1,838. The median household size was 6.8 members. The food budget exceeded 50% of the income in 47% of the families. Only 14% of the families participated in the food stamp program. Sixty-three percent of the families were paying for the school lunches of the children. Half of the children were found to be below the 25th percentile for height and weight on the Stuart anthropometric charts. Twenty-eight percent of the children up to three years of age had hemoglobin levels below 10 grams per 100 ml; 25% of the children between the ages of

TABLE 8

INFORMATION FROM FAMILY INTERVIEWS REGARDING BUDGET, HOUSEHOLD SIZE AND FOOD CONSUMPTION

Primary Sample		No. of Adequate Family Interviews
Median annual family income	$1838.	130
Median annual income per capita	$ 334.	130
Median household size	6.8 members	130
Median ratio of household members over 6 years to those under six	2.9	130
Secondary Sample		
Estimated food budget exceeding 50% of income	47%	38
Participation in Food Stamp Program	14%	51
Family pays for school lunches of sibling	63%	41

From Zee, *et al.*, St. Judes Children's Research Hospital, Memphis, Tennessee. (In press).

three to six years had hemoglobin levels below 11. In 57 randomly selected preschool children, bone age was less than 75% of the chronological age in 27%. The plasma vitamin A level was below 20 micrograms per 100 ml in 45% of the children (Table 9).

The National Nutrition Survey of certain states is turning up similar data. While most of the data are not available for public release, I was able to obtain the following information:

Ten states have been included in the survey. They are Texas, Louisiana, New York, Kentucky, Michigan, California, Washington, South Carolina, West Virginia, and Massachusetts. The survey is designed so that the first phase will place priority on low income areas. A random selection was made from Bureau of Census Enumeration Districts where the largest percentage of families were living in poverty. The estimated sample size for the 10 states will include approximately 70,000 individuals. The majority of the families falling within the sampling technique have an annual income of less than $3,000.

Some of the biochemical data have been analyzed for five of the states. Fifteen percent of the individuals examined have less-than-acceptable levels of hemoglobin; 17% have less-than-acceptable levels of serum albumin; 13% have less-than-acceptable levels of serum vitamin A; 16% have less-than-acceptable levels of vitamin C in the serum; 19% have less-than-acceptable levels of urinary riboflavin; and 9% have less-than-acceptable levels of urinary thiamine. In some of the states there is also a five-percent goiter prevalence.

There is evidence of growth retardation, since the median for the children examined thus far is equivalent to the 16th percentile on the Iowa growth charts.

Additional biochemical data from the National Nutrition Survey show adolescents with unacceptable levels for serum vitamin A, serum vitamin C, hemoglobin, urinary thiamine excretion, and urinary riboflavin excretion. Significant numbers of teenagers are biochemically malnourished, by these parameters, in the 10-to-12-year age group and in the 13-to-16-year age group. Further, there are appreciable numbers of individuals 60 years and older who have less-than-acceptable levels of vitamin A, vitamin C, hemoglobin, and serum protein.

IGNORANCE vs. POVERTY

There can be no doubt that people in all walks of life need nutrition education. Recently a food company installed hidden cameras in supermarkets in the Roxbury ghetto of Boston and in Cambridge, Massachusetts where Radcliffe graduates do most of their shopping. In Roxbury, the average housewife making one trip around the store spent $3.50 for a cart of food. The assortment of food that she purchased could not be considered a well-balanced adequate low-cost diet. In Cambridge, the Radcliffe ladies spent $9.50 per cart and purchased a better

TABLE 9

SECONDARY SAMPLE: CLINICAL AND BIOCHEMICAL INFORMATION ON 57 RANDOMLY SELECTED PRE-SCHOOL CHILDREN

Abnormality	Incidence
Bone age less than 75% of the chronological age	27%
Positive sickle cell preparation	12%
Plasma vitamin A below 20 mcg per 100ml	45%
Eosinophilia (more than 5% of the WBC)	36%
Mild upper respiratory or skin infections	25%

From Zee, *et al.*, St. Judes Children's Research Hospital, Memphis, Tennessee. (In press).

diet. The housewives from Roxbury were then given an extra six dollars and told not to hoard but to buy whatever they liked. They then proceeded to buy exactly the same things the women from Radcliffe bought.

This is not surprising in view of the fact that nutrition education is poorly taught in all of our schools, and the little information that people get about which products are more nutritious than others they receive through the media of television. The Roxbury study shows that, granting the inadequacy of nutrition education programs for all of our people, the poor can do as good a job at purchasing an adequate diet as the better-educated if they have the money to do so. This is another way of saying there is less room for error on a low income budget.

Many of us have now come to realize that the amount of nutrition education necessary to purchase an adequate diet, spending five dollars per person per week or $1,200 per year for a family of four, is probably at the graduate school level requiring at least a Master's degree. There is some evidence from the U.S.D.A. 1965 Household Food Consumption Survey that the poor get greater average returns in calories and nutrients per food dollar than those with higher incomes (Table 10).

PROSPECTS FOR THE FUTURE

I am not optimistic about prospects for the future. There is evidence that the present administration is studiously neglecting the problem. They are doing just enough to keep the heat off. Our present national priorities will not allow the allocation of sufficient funds to deal with the problem. In addition the present federal food programs, *i.e.*, food stamps and surplus food commodities, may not work even if you do pour more money into them.

In all of *our* studies we have until now concentrated too much on the victims of hunger and malnutrition. We have not begun to look at the victimizing process. It is my opinion that we have been studying the wrong people to get answers to the question of how to eliminate hunger and malnutrition. We don't know anything about how people perceive their poverty and their sense of need, the relationships between some of the uncivil servants who run the food programs and the individual recipients of these programs.

We need to know more about the political and economic circumstances in a particular county and the attitudes of local government officials which cause them to take a position against setting up a good stamp program or a surplus commodities program or cause them to administer with little or no vigor the programs that they do have.

The priorities of the Congressional agricultural committees and the U. S. Department of Agriculture at the national level favor continuing price supports and the taking of lands out of

TABLE 10

Nutrients furnished by a dollar's worth of food, in households in the United States by income (spring 1965). Low-income households had greater returns in calories and nutrients per food dollar, on the average, than households with high incomes.

Income	A dollar's worth of food provided:				
	Food Energy	*Protein*	*Calcium*	*Vitamin A value*	*Ascorbic acid*
	Cal.	*Gm*	*Mg*	*I.U.*	*Mg*
Under $3,000	3,150	99	1,090	6,860	85
$3,000 to $4,999	2,860	92	970	6,320	80
$5,000 to $6,999	2,570	85	890	5,990	81
$7,000 to $9,999	2,380	79	830	5,320	80
$10,000 and over	2,100	72	750	5,180	82

production over direct distribution of food to the needy. These priorities seem impossible to change through discussions. Moreover, high officials in the White House have diverted attention away from the nutrition problems of the poor and concentrated more on determining how well-fed the vast majority of Americans are (again an attempt to make important decisions on the basis of a public opinion poll).

We do not have the answers to these problems and to many others. Nor can we get the money to study them because of local and national politics. I would therefore not encourage hungry people that they will be fed soon.

Instead, I see a continuation of *half-hearted* efforts to establish food programs in counties which do not now have them; to spend a little more money to expand the National School Lunch Program (but none to prevent this money from being diverted to other school uses as is now the case in some areas); some experimentation with giving vouchers to pregnant women and infants and children so that they may purchase milk and infant formulas; some experimentation with the feeding of the elderly and families in school lunch rooms; and other highly visible maximum-impact pacification programs. I also see possible the enactment of the President's Welfare Program which will give $1,800 to a family of four. This will be a big improvement in many areas in the South, assuming that the county officials allow the people to have this income supplement. Apart from these changes, I can see us continuing to rock along in the same old fashion. If this is the case, then you can rest assured that the malnutrition uncovered in the National Nutrition Survey will not be treated, and the hungry will remain hungry. It seems incredible that in 1970, the American people and their government lack the will to put an end to the cycle of poverty, hunger, malnutrition, and despair from which many of our citizens seem unable to extricate themselves. The reasons why we lack the will are in themselves worthy of study. Unless we find the answers, they may spell our downfall.

James P. Carter, M.D., Dr.P.H., was one of the first to call attention to the problems of feeding low income groups in the U.S.A. He serves as a member of the National Council on Hunger and Malnutrition in the United States. He is also interested in developing new techniques of teaching social and preventive medicine to medical students. Dr. Carter is assistant professor of pediatrics and nutrition at Vanderbilt University School of Medicine and also holds an appointment in the Department of Family and Community Health at Meharry Medical College, Nashville, Tennessee. He received an M.D. from Northwestern University in 1957 and the Doctorate of Public Health from Columbia University in 1966. He is well known for his studies in tropical medicine and nutrition which were done in Egypt, Ethiopia, Uganda, the Congo, and Nigeria, as well as in the U.S.A. He has published numerous papers on protein-caloric malnutrition (kwashiorkor), zinc deficiency, and on the interrelations between malnutrition and parasitic infestation.

RECENT ADVANCES IN PERITONEAL DIALYSIS

Donald G. Vidt

Peritoneal dialysis has enjoyed widespread increase in popularity for the treatment of renal failure and a variety of other conditions. It provides the advantages of a relatively simple technic, readily-available solutions and equipment for administration, requires a minimum of the physician's time, and can be performed by a nurse or other allied medical person trained in the procedure. Peritoneal dialysis requires no permanent installation of equipment and can be performed at any convenient location in the hospital. The usual acceptable indications for peritoneal dialysis are listed in Table 1.

Despite the increasing popularity of peritoneal dialysis, it does have certain distinct disadvantages when compared with hemodialysis. Peritoneal dialysis is considerably less efficient, requiring several times as long to accomplish the same or similar removal of toxic metabolites. The use of commercial solutions and the equipment for their administration, together with the time and nursing personnel required to perform the procedure make peritoneal dialysis considerably more costly than hemodialysis. These factors, plus the discomfort associated with repeated placement of dialysis catheters and the risk of peritoneal irritation or bacterial contamination during the procedure, have limited the patient's acceptability of peritoneal dialysis. If peritoneal dialysis is to continue in favorable regard and if this procedure is ever to be considered a comparable alternative to hemodialysis, significant advances in peritoneal dialysis must be made in several directions: (1) ease of administration, (2) cost of the procedure, and (3) overall efficiency.

Ease of administration. The standard methods for peritoneal dialysis require that commercial dialysis solutions in one- or two-liter containers be administered through a percutaneously inserted plastic catheter. Solutions are allowed to remain in the abdomen for variable periods of time and are then drained by gravity into a container on the floor or below the patient's bed level. The number of cycles that comprise a dialysis and the frequency of dialysis are determined by the clinical status of the patient and the indication for which the procedure is being performed. The patient's acceptability of peritoneal dialysis is limited by the need for repeated percutaneous puncture and the significant occurrence of peritoneal irritation or bacterial infection.

A number of prosthetic devices have been designed to provide easier access to the abdominal cavity for repeated dialysis.[1-7] Their use unfortunately has been associated with an overwhelming incidence of peritonitis or fibrotic occlusion

Table I
INDICATIONS FOR PERITONEAL DIALYSIS

Acute renal failure
Chronic renal failure
Reversible deterioration
Preparation for transplantation
Long-term management
Renal failure of undetermined etiology
Refractory edema
Severe electrolyte imbalance
Intractable heart failure
Intoxication with dialyzable poisons
When hemodialysis is not possible

of the conduit. These complications have greatly limited the use of prosthetic access devices in association with the procedure.

A catheter replacement method recently described by Jacob and Deane[8] appeared to afford the ease of a prosthesis without the subsequent complications. The prosthesis consists of a solid Teflon bar connected to a mesa-shaped head.* At the termination of dialysis the prosthesis is inserted into the catheter tract and left in place until the next dialysis (Figs. 1A and 1B). The sinus tract thus established from the exterior to the peritoneal cavity provides for easy insertion of a No. 11 French catheter before each subsequent dialysis. My colleagues and I, at the Cleveland Clinic, have recently reviewed our own experience with this prosthesis, and find that it greatly improves the patient's acceptance of peritoneal dialysis. In 32 patients, with this prosthesis in place for a total of 1,441 patient-days, only three instances of bacterial peritonitis developed. There was no difference in the incidence of peritonitis whether or not prophylactic antibiotics were used. Once the sinus tract has been established, subsequent catheters may be inserted without the need for local anesthesia. We have found the use of this device to be associated with few problems, and believe that it has resulted in improved acceptance by the patient who requires repeated peritoneal dialysis. The prosthesis has been left in place for as long as 30 days between dialyses with no increased incidence of infection and remarkably little skin reaction around the prosthesis. The ease of catheter placement is such that less time from the physician is required and, in fact, there is little doubt that safe catheter placement could easily be accomplished by clinical corpsmen, nurses or other paramedical persons trained in the procedure. Skin bleeding is no longer a problem once the fistulous tract is developed; drainage problems during dialysis have been fewer; and the dangers of perforation of viscus are greatly reduced since the catheter usually can be placed without the need for a sharp stylet.

*Available from Bio-Medical Systems, Inc. Danbury, Connecticut.

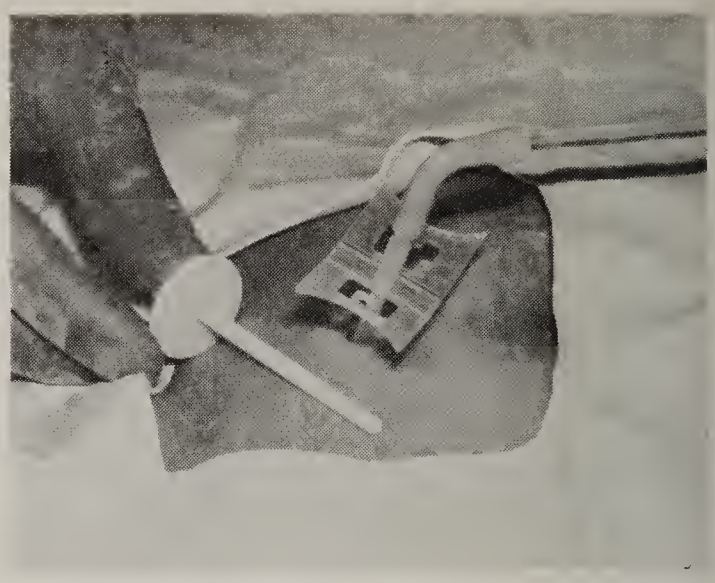


Fig. 1A.—The peritoneal replacement prosthesis is made ready for insertion just before the dialysis catheter is removed. The Teflon bar may be lubricated with anti-bacterial ointment.

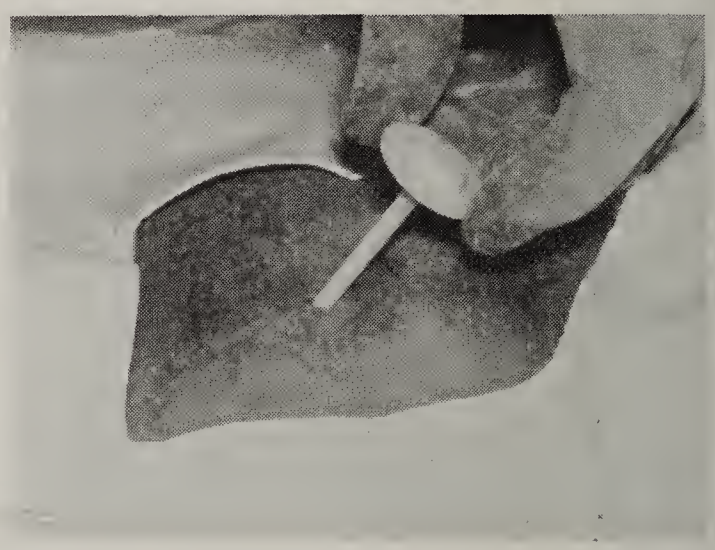


Fig. 1B.—After removing the dialysis catheter, the peritoneal replacement prosthesis is immediately inserted into the catheter tract and advanced until the head of the prosthesis is flush with the skin. It is taped in place until the next peritoneal dialysis.

Repeated puncture dialysis, even with the aid of a peritoneal access device or conduit unfortunately does not lend itself readily to home application, a seemingly necessary trend if costs are to be reduced for the procedure. For home peritoneal dialysis, an implantable device for safe, intermittent administration of dialysis solutions would seem indispensable. It must, however, be bacteriologically safe, mechanically reliable, and must be tolerated by the patient both physically and psychologically. Several catheters have

been developed which combine the advantages of a soft silastic catheter with Dacron cuffs[9] or skirts[10] designed to close the sinus tract around the catheter against bacterial intrusion. Bleeding and leakage around the catheter are eliminated, and the cuffs or skirts provide tissue fixation that prevents the catheter from being inadvertently pulled out. These silastic catheters may be inserted under local anesthesia straight into the peritoneal cavity by means of a specially designed trocar through a stab incision. The Dacron cuff or skirt is positioned just outside the peritoneum. The catheter may exit in straight fashion or may be diverted through a subcutaneous tunnel to exit through the skin at a site several inches removed from the point of entry into the peritoneal cavity. If a second cuff is used, it is positioned just beneath the skin at the site of exit of the catheter. Such a method of safe, intermittent access to the peritoneal cavity is essential for unattended home peritoneal dialysis. In combination with sterile dialysate in large containers (discussed later), this represents an important step towards the elimination of peritonitis during repeated dialysis. The present method of using one- or two-liter containers requires multiple hook-ups for a single dialysis, each offering the risk of bacterial contamination.

Cost of the Procedure. The present cost of peritoneal dialysis in the general hospital is considerable. An estimate as high as $500 for a single dialysis may be obtained when one adds together the cost of from 40 to 48 liters of dialysate, administration sets, medications and laboratory studies, hospital room, nursing personnel for two or three shifts, and professional fees when applicable. Costs may be reduced by any of several methods: the manufacture of low-cost sterile dialysate in large containers, reduction in nursing care requirements during dialysis, and development of home peritoneal dialysis for selected patients.

Several investigators have reported methods for the preparation of sterile dialysate in large containers for a fraction of the cost of a similar volume of commercially prepared dialysate purchased in one- or two-liter bottles.[11–13] The most sophisticated system is that reported by Tenckhoff and associates[12] for the preparation of 40-liter carboys of dialystate from pyrogen-free water and reagent-grade chemicals. The key to this system is a solution sterilizer fitted with a showering device that effectively shortens the exposure of solutions to high temperature, thus preventing carmelization of dextrose-containing solutions. Filtration methods are also available which use bacterial filters for sterilization, after which the dialystate is stored in pre-autoclaved carboys until used. The manufacture of peritoneal dialysis fluid in large containers not only reduces costs but allows maintenance of a closed sterile system throughout the procedure and eliminates a major source of peritoneal infection. It has also encouraged the development of automatic cycling devices which minimize the need for constant supervision of the procedure by nursing personnel.

For the smaller community hospital performing an insufficient number of dialyses to warrant the manufacture of peritoneal dialysis fluids, other simple innovations may be undertaken to minimize required nursing supervision of the dialysis procedure. We, at the Cleveland Clinic, have previously reported two simple mechanical innovations applied to commercially available administration sets.[14] A 250 ml Solu-set* may be modified to connect to the standard dialysis administration set (Fig. 2). The Solu-set valve closes automatically after two liters of dialysis solution have been delivered into the peritoneal cavity. A commercially-available Fenwall Automatic Donor scale (B. G. -2A) was modified to hold a dialysis bottle from one end of the lever arm, and a suitable counterweight from the other end (Fig. 3). When the bottle is full, the balance swings down to pinch shut the drainage tubing, and a mercury switch circuit control sounds a

*Available from Abbott Laboratories, North Chicago, Illinois.

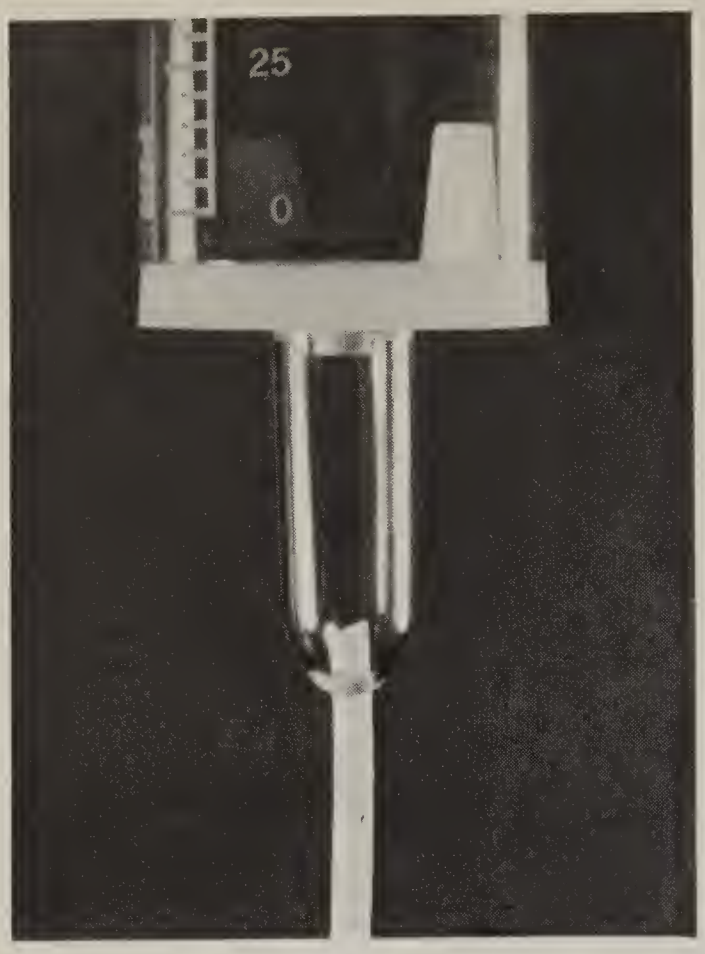


Fig. 2.—A 250-ml Solu-set is connected to a standard dialysis administration set. Closure of the flutter valve provides an automatic shut-off after two liters of dialysis solution have been delivered into the peritoneal cavity.

buzzer to signal the nurse back to the bedside. Thus, the nurse is spared the need to focus on mechanical tasks, and her attention to the patient is required only at the critical points of inflow and drainage in the dialysis procedure.

An automatic cycling machine designed for use with commercial dialysis fluids can greatly reduce the need for nursing care during the procedure, and controlled timing of cycles will reduce the total time required for dialysis. Several models currently available, although quite sophisticated in their operation, are, unfortunately, prohibitively expensive. I believe that a definite need exists for an economically-priced cycling device suitable for use with commercially-prepared dialysis solutions in one- or two-liter containers, which will provide accurate and automatic timing of cycles plus a method for warming solutions to body temperature.

Most centers have discontinued the routine addition of prophylactic antibiotics to dialyzing solutions. If careful asepsis is practiced with insertion of the catheter and during the multiple hook-ups required throughout the procedure, the incidence of peritonitis is little different whether or not the solutions contain an antibiotic in small concentration. Except for patients receiving digitalis preparations, we have discontinued the addition of potassium to our solutions without noting any significant potassium depletion, even with repeated dialyses. The only medication regularly added to solutions in our institution is heparin in a concentration of 5 mg per liter. It is our belief that this addition reduces fibrin-plugging of the catheter, particularly during insertion of the catheter. Some reduction in cost has been accomplished by the discontinuance of the above mentioned medications, without jeopardizing the safety or effectiveness of the procedure.

Home peritoneal dialysis for selected patients with chronic renal insufficiency remains the goal of many researchers. By combining the use of implanted silastic catheters with bulk solutions and an automatic cycling device, Tenckhoff and associates[15] have maintained 19 patients

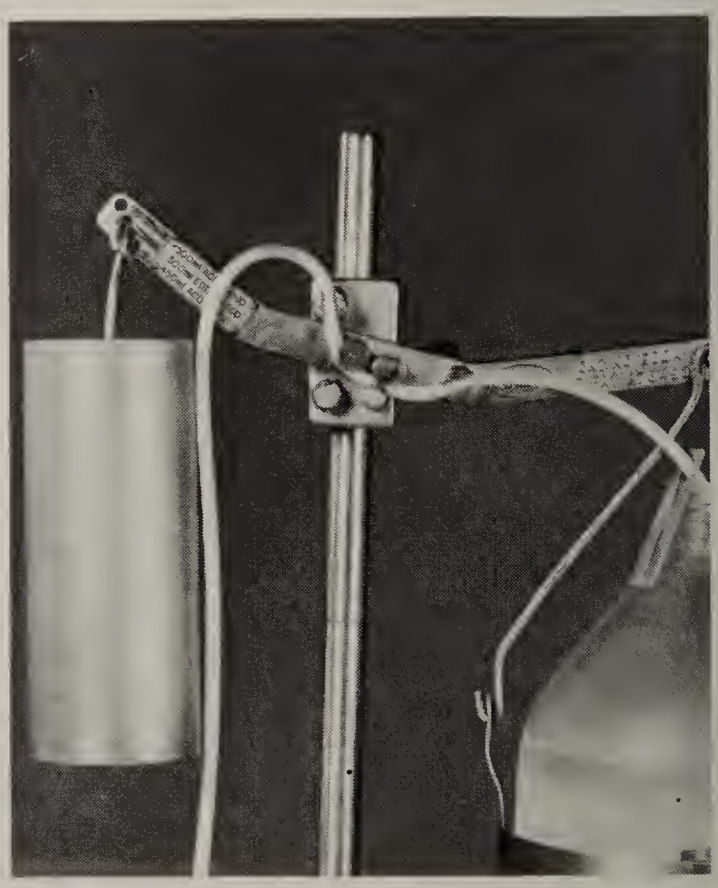


Fig. 3.—A one- or two-liter dialysis bottle has been mounted on one end of the lever arm of a Fenwall Automatic Donor Scale and a suitable counterweight on the other end.

on home peritoneal dialysis. Experience with more than 5,000 dialyses has been associated with a 0.53-percent incidence of peritonitis, despite the fact that the entire procedure is performed at home by the patient. Overnight 80-liter dialyses are performed three times weekly with three-quarters of the patients being rehabilitated sufficiently to return to work. A major problem with this program has been the manufacture and delivery of large containers of sterile dialysate to patients' homes.

Currently under evaluation is a new machine* capable of producing from tap water, 80 liters of sterile, pyrogen-free water. When mixed proportionately with sterile concentrate, a dialysate of the desired concentration is produced and delivered by an automatic cycling machine. At present, this system promises to make a home-care program possible for selected patients, at an operating cost comparable to hemodialysis performed in the home.

Overall Efficiency of Peritoneal Dialysis. Although an effective method for removal of endogenous poisons, peritoneal dialysis has the disadvantage of clearing these substances slowly and thus makes the procedure of questionable value in suicidal or accidental drug ingestions. The peritoneal clearance of endogenous toxins is affected by temperature[16] and by the use of hypertonic dialysis solutions,[17] increased clearances in the latter resulting from solvent drag plus a possible increase in membrane permeability.

Unfortunately, prolonged use of hypertonic solutions may be associated with excessive extracellular fluid volume depletion, or, in diabetic patients, may produce dangerously high blood sugar concentrations. Sorbitol, sometimes used as a substitute for dextrose, may also be associated with central nervous system symptoms if used repeatedly in concentrations exceeding 2.0 gm per 100 ml. Intraperitoneal tris-hydroxymethyl aminomethane (THAM)[18] or albumin[19] have been shown to increase barbiturate removal, but clearances rarely exceed 10 ml per minute.

Early studies by Boen[21] indicated that peritoneal urea clearances could be enhanced by increasing dialysate flow rates up to 3.5 liters per hour. The variability in clearances at any specific flow rate may relate to factors such as the surface area exposed to exchange, temperature of the fluids, and vascularity of the peritoneum. More recent studies by Tenckhoff and associates[22] have provided evidence showing two- to-threefold increases in peritoneal clearance with flow rates up to 12 liters per hour. Rapid flow rates are well tolerated, and the development of low cost dialysate in large containers will undoubtedly make dialysis at high flow rates a more feasible procedure.

Shinaberger and associates[20] have described a method of peritoneal extracorporeal recirculation dialysis which permits effective delivery of dialysis solutions at high flow rates. This is accomplished by the continuous recirculation of a small volume of sterile dialysis solution from the peritoneal cavity through an extracorporeal dialyzer, and accomplishes a significant increase in peritoneal clearances of urea and creatinine. The closed system minimizes the risk of bacterial contamination and reduces protein losses that regularly occur with peritoneal dialysis. Potential disadvantages would seem to be the requirement of two indwelling catheters, an extracorporeal dialyzer, and the seeming need for closer supervision of such a system.

At present, attempts to improve the transport of substances across the peritoneum have been limited to adjustments in pH, temperature, flow rates, changes in the osmotic gradient, or binding of substances in dialysis fluid. These methods all involve physical changes in the solution or solute without affecting the peritoneal membrane itself.

If permeability of the peritoneal membrane could be altered by chemical agents, it might be possible to increase the transport of certain substances across the membrane. In a search for agents to accelerate dialysis across the peritoneum,

*Cobe Laboratories, Inc., Denver, Colorado.

a number of wetting agents have been tested and used experimentally in the peritoneal fluid. Tris-hydroxymethyl aminomethane (THAM) demonstrates weak surface-active properties and modestly accelerates peritoneal dialysis by an unknown mechanism. Penzotti and Mattocks[23] have experimentally tested a group of common wetting agents, including some anionic, cationic, and nonionic compounds. While the nonionic wetting agents showed little acceleration, the cationic wetting agents, although effective accelerators, proved too toxic at the levels tested. The most effective anionic agent tested was dioctyl sodium sulfosuccinate (DSS) which appeared to accelerate the dialysis rates of urea fourfold, and provided a two- to-threefold increase in dialysis of creatinine. Fig. 4 demonstrates the degree of acceleration seen with dialysate containing an effective wetting agent, DSS, in comparison with that of the control fluid. It is also possible that DSS or similar agents might accelerate the removal of drugs such as salicylates or barbiturates, and thus make peritoneal dialysis a more aggressive mode of therapy for selected intoxications. Before these agents can be evaluated in man, extensive experimental evaluation of their absorption and accumulation in the body must be undertaken and correlated with possible toxicity, particularly in circumstances of impaired renal function.

ACCELERATION OF PERITONEAL DIALYSIS BY SURFACE-ACTIVE AGENT (DSS)

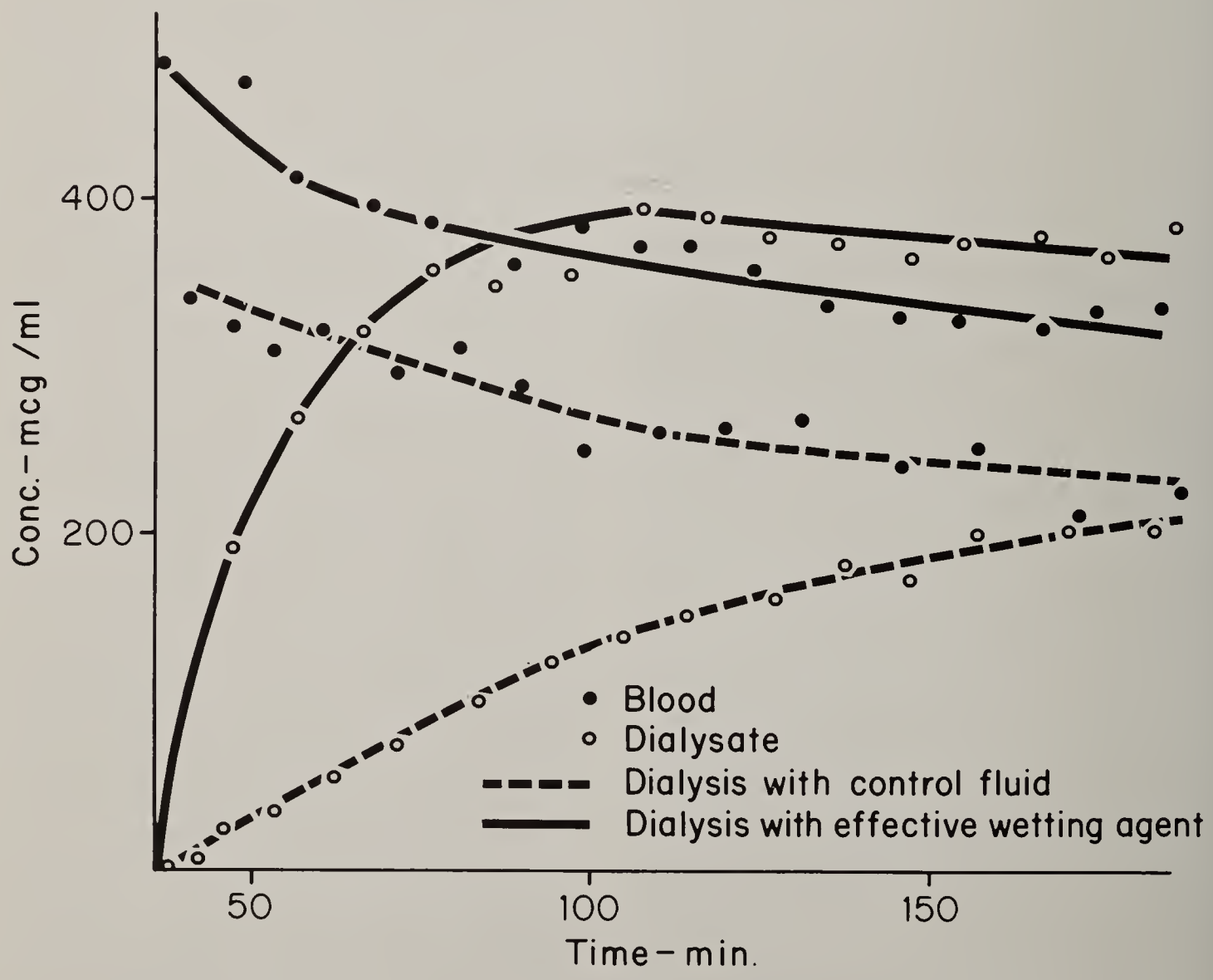


Fig. 4.—Using C^{14}-tagged urea, the accelerating effect of dioctyl sodium sulfosuccinate (DSS) on peritoneal clearance can be demonstrated.

SUMMARY

Newer developments in the procedure of peritoneal dialysis have been reviewed. Developments in administration equipment and packaging of solutions will improve acceptance by patients requiring the procedure, and will reduce costs. Attention must also be focused on means of improving the efficiency of peritoneal dialysis if this procedure is ever to be considered a true alternative to hemodialysis in the treatment of selected patients with renal failure.

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Donald G. Vidt, M.S., M.D., is known for his work on the kidney, particularly as it relates to treatment of chronic renal failure by dialysis or transplantation. In addition, he is an authority on hypertension and has published a number of papers on his observations. Dr. Vidt is a member of the staff of the Department of Hypertension and Renal Diseases at the Cleveland Clinic, Cleveland, Ohio. He studied physiology at Seton Hall University, at New York University, and at Ohio State University, obtaining the M.S. in physiology in 1954 and the M.D. in 1959 from Ohio State University.

RENAL SYNTHESIS OF AMMONIA AND ITS IMPLICATIONS*

George A. O. Alleyne

Urinary excretion of ammonia serves as an important mechanism for sparing cations as well as for eliminating hydrogen ion from the body. The free base NH_3 is lipid-soluble and lacks electrical charge, so it readily diffuses across cell membranes and tends to equilibrate in renal tubular cells, tubular urine, and peritubular blood. In contrast, the ammonium ion NH_4^+ is trapped wherever it is formed. This process of ionic diffusion and trapping is the mechanism by which almost all, if not all, ammonia is excreted into the urine.

Since the renal excretion of ammonia is determined mainly by the renal cellular production of ammonia, emphasis is being placed now on the mechanisms which initiate and control ammonia production. Mainly through the classical work of Pitts and his collaborators, it has been established that glutamine accounts for 90% of that ammonia formed in the kidney. This glutamine may be metabolized (Fig. 1) either by deamidation (glutaminase I) or by transmination and subsequent deamidation (glutaminase II—or glutamine transaminase + ω-amidase). It has also been shown under conditions of metabolic acidosis that there is enhanced renal gluconeogenesis in the rat, and it has been postulated that this serves to remove the glutamate formed as a result of glutamine deamidation, since glutamate inhibits glutaminase I.

My experiments have been directed towards the control of the gluconeogenesis and ammoniagenesis in the rat. Rats were made acidotic by feeding ammonium chloride for 48 hours; the kidneys were then removed and key metabolic intermediates measured. Compared with controls, there was a fall in levels of citrate, α-ketoglutarate and malate, but a rise in phosphoenolpyruvate. This was interpreted as indicating an increase in activity of phosphoenolpyruvate carboxykinase (PEPCK), the enzyme which effects the decarboxylation of oxaloacetate with resultant formation of phosphoenolpyruvate. This was confirmed when PEPCK was measured in the renal cortex of acidotic rats and it could be shown that there was an early increase in activity. PEPCK activity was related to the degree of acidosis and the level of urinary ammonia.

In vitro experiments with renal cortical slices from rats made acidotic for varying periods showed that an increase in glucose and ammonia production was demonstrable in slices from rats fed acid just two hours previously. Glucose production rose progressively with increasing duration of acidosis, but in the early stages of acidosis there was a disproportionate increase in ammonia production. Metabolic intermediates were next measured in kidneys from rats acidotic for the times shown (Fig. 2). There was an immediate fall in malate and α-ketoglutarate and a rise in aspartate. At six hours there was a significant increase in phosphoenolpyruvate, which had increased even further by 48 hours. Measurement of PEPCK at these times showed that there was already a significant increase in

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**The Joseph Davis A. Memorial Lecture, presented at the Program to Inaugurate New Facilities for the Section of Renal Diseases and Nutrition, Department of Medicine, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois, April 19, 1970.*

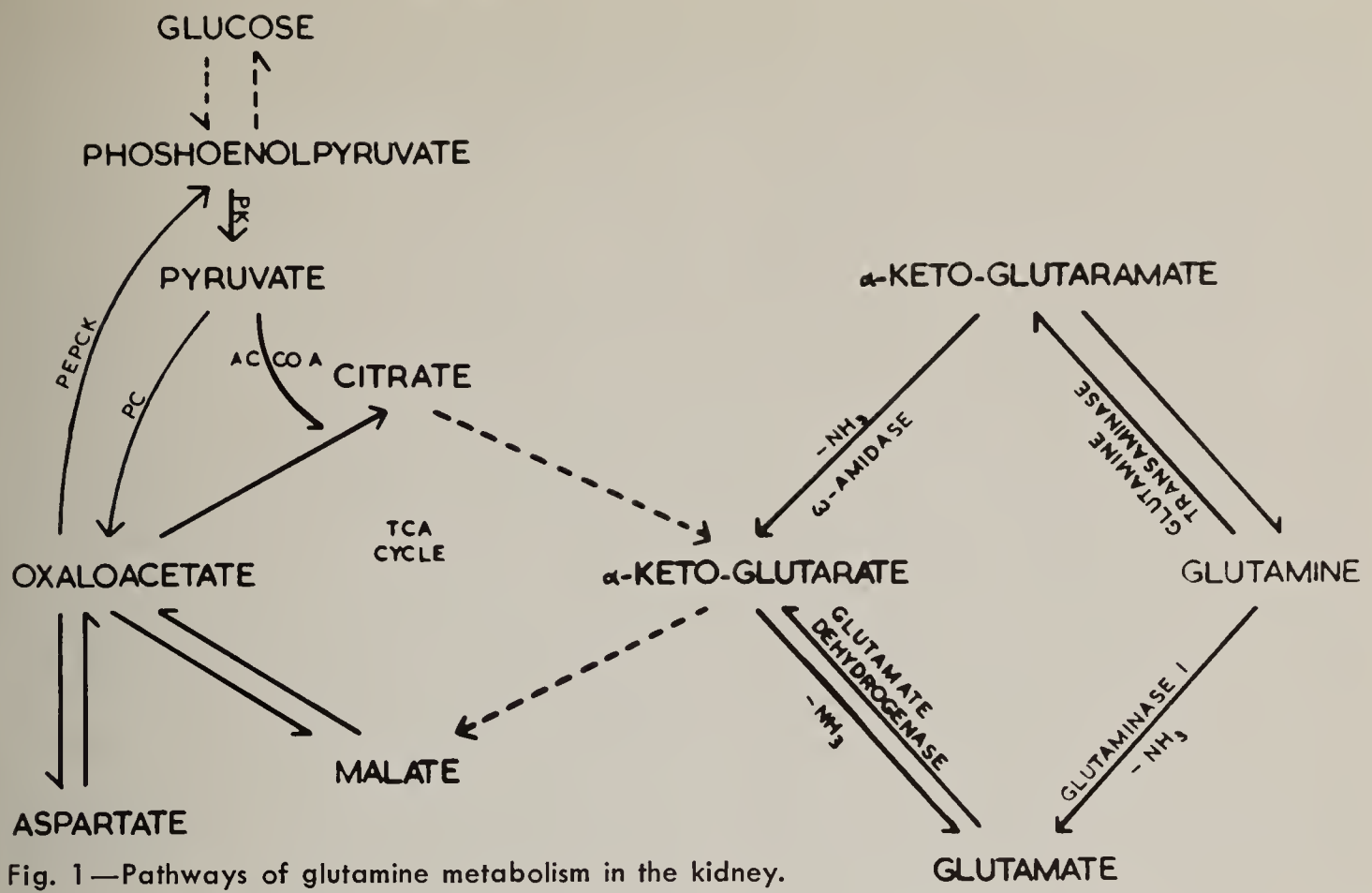

Fig. 1—Pathways of glutamine metabolism in the kidney.

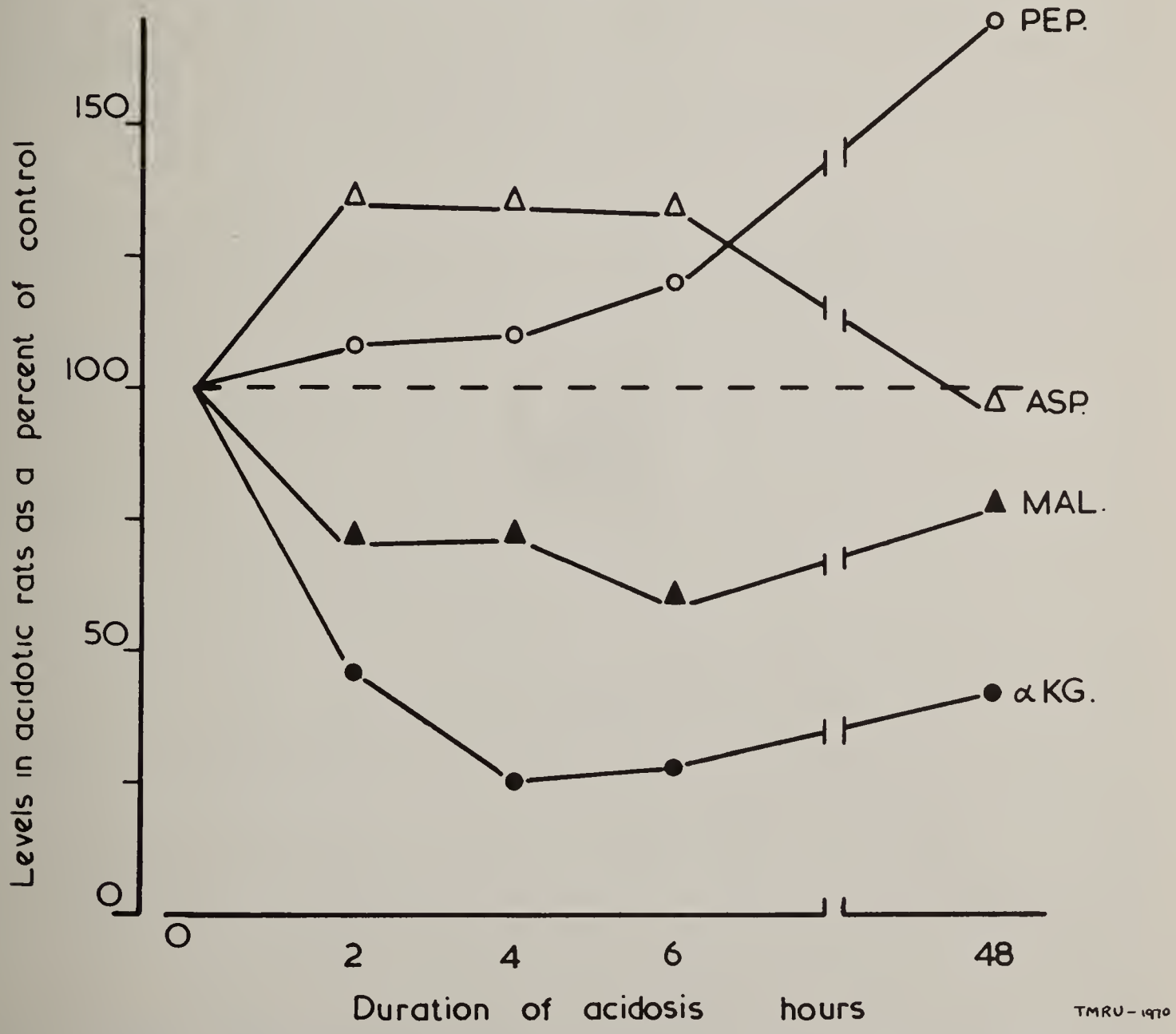

Fig. 2.—Changes in metabolic intermediates phosphoenolpyruvate (PEP), aspartate (ASP), malate (MAL), and α-ketoglutarate (α-KG) in response to acidosis.

activity four hours after an acid load. Ammonium chloride was next fed to rats, and urine collected at half-hour intervals. There was a significant increase in urinary ammonia one and one-half hours after administration of the acid load.

The disproportionate increase in ammonia production over glucose production early in acidosis is interpreted as showing increased activity of the glutaminase II system.

In order to depict more clearly the interrelationship between glucose and ammonia production, glucose production was stimulated by administration of steroids *in vivo*. In spite of a mild metabolic alkalosis, there was an increase in ammonia production. PEPCK was measured in the kidneys of rats treated with steroids and found to be increased. When steroid-treated rats were fed ammonium chloride there was a further increase in urinary ammonia and cortical PEPCK activity.

Conversely, gluconeogenesis was inhibited by administration of phenylethylbiguandide (PEBG), and a decrease in urinary ammonia resulted. Rats were given PEBG intraperitoneally, NH_4Cl orally or PEBG + NH_4Cl and sacrificed two hours later. NH_4Cl produced the expected increase in glucose and ammonia production in cortical slices, but this was blocked in the PEBG-treated rats. *In vitro* experiments with PEGB showed that with increasing concentration of the drug, there was a progressive decrease in production of ammonia and glucose.

The data thus show that a characteristic metabolic pattern develops in response to a metabolic acidosis. In the early stages of acidosis there is probably increased activity of glutaminase II, but with progressive acidosis and increased production of ammonia, there is increased gluconeogenesis, and, certainly in the chronically acidotic rat, there is a close link between gluconeogenesis and ammonia production.

George A. O. Alleyne, M.D., M.R.C.P., has published many papers dealing with malnutrition, particularly protein-calorie malnutrition. In addition, he has made many contributions to the study and treatment of renal disorders. Recently he has been particularly interested in defining details of the production of ammonia by the kidney. Dr. Alleyne is a member of the scientific staff of the Medical Research Council Tropical Medical Research Unit, and is also Associate Dean of the Medical School, University of the West Indies, Kingston, Jamaica. Dr. Alleyne was educated in Barbados and Jamaica. He was graduated from the University of the West Indies in 1957. In 1961-64, he was a Glaxo Fellow and worked at University College Hospital, London, with Sir Max Rosenheim. In 1963, he was appointed to the scientific staff of the Medical Research Council. He is also an honorary senior attending physician to the University Hospital in Mona, Jamaica. In 1967, he spent his sabbatical leave working with Dr. Arnold Relman at Boston University Medical School. He is a member of the Royal College of Physicians of London and is also M.D., London University.

PROSTAGLANDINS: THE STATUS OF THEIR UTILITY IN CARDIOVASCULAR-RENAL AND OTHER DISEASES

James R. Weeks

Over 35 years ago, U. S. von Euler and Maurice Goldblatt independently described an unusual vasodepressor, smooth-muscle stimulating principle in human semen and in the semen and vesicular glands of the sheep.[1,2] We now know that this principle, named "prostaglandin," consists of a family of fatty acids. They are widely distributed in mammalian tissues and, in many biological systems, are among the most potent substances known. Indeed, the variety of their potent pharmacological actions makes them very fascinating research subjects but, on the other hand, makes a rational explanation of possible physiological roles very difficult. In addition to stimulating smooth muscles and lowering blood pressure, certain prostaglandins can affect female fertility, inhibit gastric secretion, inhibit platelet aggregation, cause diuresis, constrict the nasal mucosa, inhibit lipolysis and mimic the effects of certain of the pituitary trophic hormones. The complete list is much longer and even more varied, so the discussion here must be limited. More detailed information may be found in recent reviews and summaries.[3–8]

I shall survey those aspects of prostaglandins which have promising practical clinical applications. If the topics seem incoherent, it is because the biological knowledge of prostaglandins is too. There is a prostaglandin "information explosion" in process, and the significant fragments have not yet been assembled. At the end of 1969, the world's technical literature on prostaglandins listed about 1200 citations, but over two-thirds had appeared in 1968 and 1969 alone!

Chemistry, Biosynthesis and Occurrence:

All the prostaglandins (PG) are unsaturated hydroxy-acids with a 5-membered ring on a 20-carbon skeleton. The commonest of the dozen-odd naturally occurring prostaglandins are prostaglandins E_1, E_2, $F_1\alpha$, and $F_2\alpha$ (Fig. 1). The E- and F-series prostaglandins differ in that the substituent on carbon-9 is either keto or α-hydroxy, respectively. The 1- and 2-series prostaglandins differ in that there are, respectively, one or two double bonds in the side chains. The corresponding A and B prostaglandins are dehydration products with an extra double bond in the ring. Among the E, Fα, and A prostaglandins, there are often both qualitative and quantitative differences. Although there are some qualitative differences between PG_1 and PG_2 prostaglandins, quantitative differences are more numerous. In nature, prostaglandins are formed enzymatically by oxidation and cyclization of the 20-carbon polyunsaturated fatty acids, *dihomo*-γ-linolenic acid for PG_1's and arachidonic acid for PG_2's. These enzymes are widely distributed in many tissues. The precursor acids are the "essential" fatty acids (EFA), but attempts to reverse signs of EFA deficiency in animals have been disappointing. Nevertheless, among related polyunsaturated fatty acids, there is a correla-

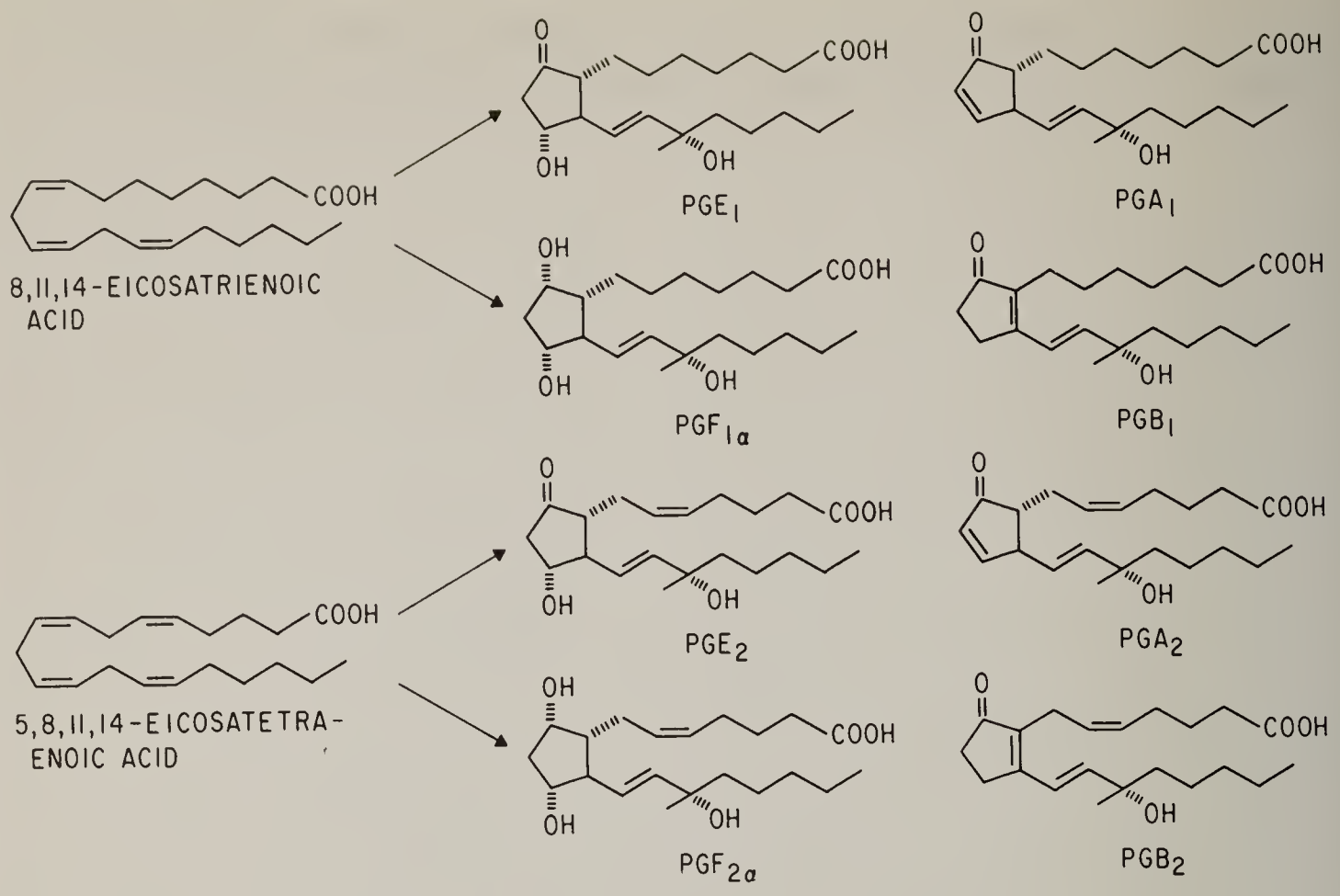

Fig. 1.—Structural formulas of selected naturally occurring prostaglandins and their precursors. 8,11,14-Eicosatrienoic and 5,8,11,14-eicosatetraenoic acids are *dihomo*-γ-linolenic and arachidonic acids, respectively. PGA and PGB prostaglandins were formerly designated PGE-217 and PGE-278, respectively.

tion between ability to reverse EFA deficiency in rats and suitability as a substrate for the converting enzyme,[9] so work continues on this logical association.

On appropriate stimulation, many diverse organs will release prostaglandins.[3] A few representative examples are: the adrenal gland, spleen, cerebral cortex, stomach, intestine, depot adipose tissue and the kidney. In this connection, we must recognize that chemical methods leading to specific identification and quantitation of prostaglandins, at the submicrogram-per-gram levels in tissues and biological fluids, are not yet available. Bioassays must still be used, and identification is presumptive, based upon selective solvent extractions and mobility in thin-layer chromatography which corresponds to standard prostaglandins. The quantities of prostaglandins released in such situations are greater than the amounts found by extraction of the tissue. Thus, tissue stores are unlikely. Instead, it is the precursor acid which is liberated, probably from a phospholipid, which then gains access to converting enzymes already present.[10,11]

A wide variety of human and animal tissues have been assayed for their prostaglandin content. However, recent studies indicate that such values may represent only the capability of tissue to biosynthesize prostaglandins in the interval between removal and preparation for extraction.[12,13] Tissues processed very quickly did not have detectable prostaglandin levels.

Promising Clinical Uses:

The promising clinical uses differ for the various prostaglandins. It is very likely that for some uses a synthetic prostaglandin analog will be more useful than one of the natural compounds. Thus, the natural compounds are poorly absorbed orally. Their duration of action is also brief due to rapid metabolism. The E and Fα prostaglandins are very rapidly inactivated by lung enzymes,[14] which

not only makes necessary administration by intravenous infusion but also argues against their being circulating hormones in the classical sense.

Uterine stimulant for induction of labor and abortion: This application is for prostaglandins E_2 and $F_{2}\alpha$, since the prostaglandins of the A series (PGA) have very little smooth-muscle stimulating activity. The smooth muscle in many organs contracts when exposed to these prostaglandins, but uterine muscle is especially sensitive. It is possible that these prostaglandins play a physiological role in labor. They are found in biologically high concentrations in amniotic fluid, but only at term.[15] During labor contractions, but not during the interval between them, a PG-like material appears in the blood.[16] Initial clinical studies have been very encouraging for induction of labor, both elective and therapeutic. Doses are very small, intravenous infusion of 50 ng/kg/min for $PGF_{2}\alpha$[17] and only a miniscule, 5 to 10 ng/kg/min, for PGE_2.[18]

Contrary to oxytocin, the prostaglandins are potent uterine stimulants in early as well as late pregnancy.[19-21] Indeed, uterine bleeding and termination of pregnancy could be induced with $PGF_{2}\alpha$ as early as the sixth to eighth week of pregnancy.[22] With the liberalization of abortion laws, the importance and utility of a non-surgical abortifacient is obvious.

Antinidatory agent for contraception: In the normal ovarian cycle, the follicle develops, matures, an ovum is discharged, and then a corpus luteum forms from certain cells present in the follicle. If the ovum were fertilized, this corpus luteum would continue to grow and secrete progesterone; otherwise, it would stop secretion and regress. The mechanism leading to regression remains unknown, but some experimental evidence points to a blood-borne factor produced in the uterus. An hypothesis has been proposed that this factor could be $PGF_{2}\alpha$.[23] Whether or not this hypothesis is true, at least $PGF_{2}\alpha$ is able to induce regression of the corpus luteum in several species. Blood progesterone falls to undetectable levels, and without this hormone, nidation of the fertilized ovum cannot take place nor can an early pregnancy be maintained. This type of contraceptive action has been observed in monkeys as well as several rodent species (see 24 for references). The potential of such a contraceptive agent is obvious. For any extended practical application, however, it would appear that an orally effective preparation would be desirable.

Bronchodilator for asthma: Prostaglandins of the E-series (PGE) relax bronchial smooth muscle, the Fα's contract, and the A's are relatively inactive. In animal tests, this relaxant activity of PGE's was not outstanding, effective doses leading to cardiovascular side effects. Then it was observed that human bronchial muscle, *in vitro*, was very sensitive to this action of PGE's.[25] Preliminary human studies have shown that an aerosol inhalation of PGE_1 had no effect on resistance to air flow in normal subjects but reduced resistance in asthmatic subjects. On a weight basis, it had about ten times the potency of isoproterenol.[26]

Gastric antisecretory for ulcers: The E and A prostaglandins, but not the Fα, when given by continuous infusion, can completely inhibit gastric acid secretion in dogs.[27] Furthermore, both gastric secretion and experimental ulcer formation can be inhibited in rats.[28] The mechanism of this action seems to be inhibition of acid formation rather than an indirect effect.[29] Results of human studies on this activity have not yet been published.

Inhibition of platelet aggregation: This action of prostaglandins is present to an appreciable degree only in PGE_1. However, PGE_1 is very potent, inhibiting aggregation of platelets induced in a variety of ways in concentrations as little as a few nanograms per milliliter. Nevertheless, applications for this action probably will be only *in vitro*, since effective *in vivo* doses also cause side effects.[30] On the basis of animal studies, uses would be in the harvesting and preservation of platelets for transfusion and preservation of isolated organs prior to transplant.[31]

Cardiovascular/Renal Actions and Hypertension:

The overall cardiovascular effect of PGE's and PGA's is that of a peripheral vasodilator, leading to a fall in blood pressure and increased cardiac output.[32] On the other hand, the overall effect of $PGF_{2\alpha}$ varies with species, being depressor in cats and rabbits but pressor in rats, dogs,[33] monkeys,[34] and, in comparable doses according to body weight, man.[35] Clinical interest in cardiovascular actions is centered about PGA_1, which shares the cardiovascular and renal effects of the PGE's but not their smooth-muscle stimulating actions. Its acute toxicity is very low and it is well tolerated by intravenous infusion in man.

Present clinical interest and potential relationships to the pathogenesis of hypertension center about the renal effects of the prostaglandins. Infusion of PGE_1 or PGA_1 into the renal artery of dogs causes increased renal blood flow, diuresis and natruresis.[36,37] Effective blood concentrations are on the order of 0.1 ng/ml. This observation alone would not be interesting, since other vasodilators, as acetylcholine, have a similar action. However, prostaglandins have been isolated from kidneys, or at least the kidney has the potential for their rapid biosynthesis.[38] Furthermore, a variety of procedures will elicit release of prostaglandin or prostaglandin-like substance; for example, renal nerve stimulation,[39] norepinephrine infusion,[40] angiotensin infusion,[41] and renal ischemia.[42] All of these procedures lead to a decreased renal blood flow, suggesting that the prostaglandin release may be a compensatory reaction. In human renal hypertension, a prostaglandin-like substance has been detected in the renal vein blood from affected kidneys only.[43] In dogs, infusion of PGA_1 increases renal blood flow with associated diuresis and natruresis in doses which do not affect systemic blood pressure.[44] This implies a selective action on the renal vasculature. In humans with essential hypertension, PGA_1 infusion lowered blood pressure, caused diuresis and natruresis, sometimes striking, but with an increase in renal blood flow.[45–48] One investigator has reported that the natruretic effect of PGA_1 was potentiated by vasopressin infusion.[47,48]

This same investigator reported that infusions of less than 0.5 μg/kg/min, which did not lower blood pressure of either normotensive or hypertensive subjects, did so in four anephric dialysis patients.

It would seem that PGA_1 would be especially useful in conditions where there is a vicious circle of hypertension and severely impaired renal circulation. Then an intravenous infusion, over a period of hours if necessary, could not only lower blood pressure but reestablish renal blood flow and allow renal function to resume.

On the basis of such animal and clinical observations, the hypothesis has been advanced that the prostaglandins may play a role in the maintenance of blood pressure, with prostaglandins formed within the kidney modulating renal blood flow and the excretion of sodium and water.[49] The computer analysis of the pathophysiology of hypertension by Guyton and Coleman implied that renal resistance was of prime importance in maintenance of blood pressure.[50] It is clear that a role for prostaglandins in hypertension is not established, but on the other hand, what we do know clearly justifies further research along these lines.

SUMMARY

The prostaglandins are a family of unsaturated hydroxy-acids with a 5-membered ring on a 20-carbon skeleton. They were originally discovered in human and ram semen over 35 years ago and described simply as unusual vasodepressor, smooth-muscle stimulating substances. In recent years, they have been shown to be ubiquitous with most mammalian tissues having the potential to form prostaglandins enzymatically from certain poly-unsaturated fatty acids. Physiological roles are yet to be definitively established, but their extreme

potency in many different living systems implies such a role. Because of rapid inactivation, especially by the lungs, a local rather than systemic function seems more probable. Several of the actions of prostaglandins indicate clinical utility; for example, uterine stimulation for induction of labor or therapeutic abortion, a luteolytic action on the corpus luteum for contraception, bronchodilatation in asthma, inhibition of gastric acid secretion in ulcers, inhibition of platelet aggregation, and renal vasodilatation, accompanied by diuresis and natruresis. The practical clinical usefulness of these compounds is under study, and preliminary results are exciting.

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James R. Weeks, Ph.D., is a research associate at Upjohn Company, Kalamazoo. He has done considerable research on hypertension. He has been interested in experimental addiction. However, he is a world authority, and is best known for his work on the prostaglandins, a group of natural lipids recently recognized to have great significance in health and disease. Educated in Des Moines, Iowa and the University of Nebraska, he was appointed a Dow Chemical Fellow at the University of Michigan where he obtained his Ph.D. degree in pharmacology in 1952. He then was appointed to the pharmacology department at Drake University and was professor in the department from 1954 to 1957. He joined the Upjohn Company in 1957 and has worked there in the field of cardiovascular pharmacology.

PATTERNS OF RECURRENCE IN CHRONIC BACTERIURIA AND DIFFERENTIATION OF RENAL FROM BLADDER BACTERIURIA

MARVIN TURCK

One possible explanation for the relative refractoriness of chronic urinary tract infections to both medical and surgical therapeutic measures is the persistence of foci of infection within the renal parenchyma. In this sense, chronic urinary tract infections and subacute bacterial endocarditis may be similar. In the latter a comparatively brief course of antibiotic therapy often does not eliminate the causative pathogen, resulting in relapse, and elimination of infection may be accomplished merely by continuation of the same antimicrobial regimen for a longer period, *i.e.*, six weeks.

Although prolonged treatment of urinary tract infections has also been advocated by several investigators, controlled studies frequently have not demonstrated any significant benefit over more conventional courses of therapy that usually last from seven to fourteen days. One reason for not achieving better results in chronic urinary tract infection, even after protracted antimicrobial therapy, has been failure to differentiate two patterns of recurrent infections—relapse from reinfection. The precise bacteriologic and serologic identification of enterobacteriaceae has permitted the definition of a relapse as a recurrence that is due to the same species and type-specific strain of micro-organism present before therapy as opposed to a reinfection which means that a new pathogen appears during or after treatment.

When these definitions were applied to a group of more than 100 patients with chronic bacteriuria, we demonstrated by ureteric catheterization that patients who had relapses with the same strain usually had infection emanating from one or both kidneys, and that these patients benefited from more prolonged treatment provided that their urine became sterile during the initial course of therapy. On the other hand, the pattern of reinfection with new strains was commoner among patients whose infection was confined to the bladder and was not altered by the duration of treatment of the individual infection. However, these observations were obtained primarily in women without structural abnormalities of the urinary collecting system who were free of other factors believed to be deterrents to bacteriologic cure. In men, lesions of the lower urinary tract *per se*, such as chronic prostatitis, also may be associated with relapsing urinary infection, and the pattern of recurrence may not be helpful in pinpointing the infection to the upper or lower tract. Nevertheless, this does not invalidate the usefulness of determining the precise pattern of recurrence in the management of patients with bacteriuria.

The studies of McCabe and Jackson[1] on the treatment of patients with recurrent bacteriuria also depict the importance of defining the pattern of recurrence in chronic urinary tract infection. These workers studied a group of 252 patients with bacteriuria and observed their course following antimicrobial therapy. Of the initial group of patients, 202 (80%) developed negative urine cultures during the first course of treatment and

were followed for their pattern of recurrence. Sixty-six (33%) relapsed with the same bacterial species and, in general, did so immediately following cessation of therapy. On the other hand, bacteriuria with a new species or strain (reinfection) occurred in 55 (27%) of these patients and was observed several weeks or months following completion of initial treatment.

We have observed also that most patients who relapse with the same strain will do so shortly after cessation of antimicrobial treatment. In these patients, extension of treatment for six weeks beyond the conventional ten to fourteen days of therapy will frequently be associated with cure provided that renal stones or marked impairment in renal function is not present. Conversely, prolonged courses of antimicrobial therapy probably are not warranted in patients with repeated reinfections with new organisms. Of course, exceptions to this general stand need to be made and occasional patients with persistent lower-tract infections may require suppression of lower-tract infections with antimicrobial regimens analogous to those used by the Cooperative U.S.P.H.S. Hospital Study. However, to date there is little evidence to support the view that prolonged treatment of patients with repeated reinfections prevents progression of renal functional deterioration. In fact, there is little evidence that these patients have abnormal renal function to begin with. In addition, not all workers in the field agree that prolongation of treatment is the optimal approach to the treatment of patients with documented relapsing renal bacteriuria. For example, Brumfitt and co-workers[2] favor intensive treatment with high doses of parenteral chemotherapeutics, and do not believe that better results are achieved by prolongation of treatment. At this writing, dogmatic statements concerning the optimal duration of treatment of chronic bacteriuria should not be made. It is anticipated that the greater availability of typing sera, coupled with a clearer definition and description of population groups studied, eventually will lead to a rational therapeutic approach to recurrent infection of the urinary tract.

The differentiation of renal from bladder bacteriuria is useful not only in defining groups of patients for epidemiologic and therapeutic studies, but also for guiding the duration and intensity of treatment in an individual patient, and an accurate method for locating bacteriuria precisely is needed. As has been mentioned, while the pattern of recurrence is helpful clinically, it is an accurate prediction in only about 80% of patients. At the present time the most reliable technique for the localization of urinary infection is bilateral ureteric catheterization. Even this procedure, however, is not absolutely predictive of the site of infection, because ureteric catheterization diagnoses ureteric bacteriuria which is not necessarily synonymous with renal parenchymal infection, but may signify infection confined solely to the renal pelvis. Despite this apparent limitation, the technique of bilateral ureteric catheterization is useful for investigating patients with well documented recurrent infection. When performed properly, it entails little discomfort or risk to the patient, and is not unduly cumbersome or time-consuming. Nevertheless, ureteric catheterization should not be taken lightly and until more data are accumulated, cannot be justified for the routine evaluation of patients with recurrent bacteriuria. Rather, the technique should be looked upon as a research tool for gathering further information concerning the natural history of urinary tract infection. Moreover, it should serve as the baseline for evaluating simpler tests designed to differentiate upper from lower urinary tract infection. More recently, Fairley and co-workers[3] have evaluated a procedure which entails bladder but not ureteric catheterization. These workers studied 71 women with recurrent urinary tract infection employing a technique of bladder wash through a bladder catheter and compared the results of these collections with results of samples obtained by ureteric

catheterization. In general, patients with documented upper-tract infection had high counts persisting in the bladder urine at the termination of the bladder wash, whereas patients with infection confined to the bladder had sterile wash specimens. Moreover, these workers observed that the wash procedure *per se* often cured bladder bacteriuria. Although this procedure is less cumbersome and may be more useful in studying large groups of patients, it has more limited investigative potential than ureteric catheterization because of its inability to distinguish unilateral from bilateral upper tract involvement. Nevertheless, it does obviate the necessity to catheterize the ureters in infected patients and deserves further study.

In the past, several laboratory tests, which have included, among others, selective staining of the urinary sediment, and observation of the sediment after intravenous administration of bacterial pyrogen or adrenal corticosteroid have been proposed as facilitating the distinction between upper and lower urinary infection. More recently, measurements of beta-glucuronidase activity in the urine, O-specific hemagglutinating and other antibody titers and maximal concentrating ability have also been put forth as methods for pinpointing renal involvement in chronic bacteriuria. Although these procedures may be helpful in analyzing groups of patients, none is specific in individual patients, and to date only bilateral ureteric catheterization has been shown to localize infection with relative certainty.

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Marvin Turck, M.D., has made many contributions to the study and treatment of infectious diseases, particularly as they relate to the kidney and urinary tract. He is a member of many academic and scientific societies. Dr. Turck is associate professor of medicine at the University of Washington, Seattle, and Chief of Medicine of the United States Public Health Service Hospital, Seattle. He was educated at the University of Illinois and graduated from the College of Medicine in 1959. After completing his residency training in Chicago, he received a research career development award of the National Institutes of Health in Seattle.

BODY COMPOSITION IN RENAL FAILURE: EFFECTS OF ALTERATIONS IN DIETARY NUTRIENTS ON BODY COMPOSITION, BLOOD PRESSURE AND RENAL FUNCTION

James C. Hunt
Ladislav P. Novak
Ralph A. Nelson

Practitioners have long recognized that subjects with renal failure may present signs of sodium or water excess or depletion, hypertension or hypotension, wasting of muscle tissue or body fat, azotemia and other disorders of water, electrolyte and protein metabolism.[1-4] Several aspects of body composition have been clarified in renal failure with edema or volume depletion through metabolic balance studies; however, simultaneous measurement of tissue wasting and alterations of body water and electrolytes and their influence on blood pressure and renal function has become accessible with the application of isotope dilution and whole-body counting methods to the study of body composition in man.[5,6]

In health, the human kidney can lower urinary sodium concentration to near zero during sodium restriction and can excrete large salt loads with little change in body water and electrolytes. With advanced renal disease, patients may suffer severe impairment of ability to conserve or excrete water and electrolytes and experience progressively more severe retention of products of protein metabolism. Multiple tracer studies in subjects with renal failure and edema have indicated changes in composition characterized by increased serum potassium, decreased total exchangeable potassium, increased total body water, extracellular water and total exchangeable sodium and decreased body cell mass and body fat.[5,6]

Careful studies as to the mechanism of salt wastage in patients with chronic renal disease have emphasized that not all patients with renal failure respond to sodium-controlled diets in a similar fashion.[4,7] Our previous studies have confirmed these observations; however, we have been impressed that patients with primary hypertension and renal failure and patients with a variety of glomerular lesions, renal failure and hypertension may react differently to carefully planned dietary regimens with control of protein, electrolytes and water and that sodium restriction may greatly improve blood-pressure control in these patients.[8,9] The purpose of this study was to undertake preliminary examination of alterations in body composition and their effects on blood pressure and renal function in patients with advanced renal failure, through control of dietary sodium, protein, water and total calories. Comparison was made between body composition values before and, when possible, after establishment of an individualized therapeutic regimen.

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SUBJECTS AND METHODS

Subjects

The group studied included twenty patients with severe renal insufficiency or failure with hypertension but without evidence of other primary systemic

disease. Among those subjects were ten patients with chronic glomerulonephritis, three with apparent primary hypertension and hypertensive renal disease, two with interstitial nephritis presumably resulting from chronic drug abuse, two with chronic recurrent bacteriuria with symptomatic pyelonephritis, two with polycystic renal disease and one with severe chronic potassium depletion and aldosteronism. One patient with chronic glomerulonephritis had evidence of angiospastic malignant hypertension, anasarca, symptomatic uremia and oliguria, and required peritoneal and subsequently hemodialysis in order to effect control of blood pressure, water and electrolyte metabolism and uremia. Three patients died in uremia during the course of treatment and one has not been available for follow-up studies. Five cases with accelerated primary hypertension, glomerulonephritis, primary aldosteronism or interstitial nephritis with renal failure and hypertension were chosen to illustrate clinical-laboratory correlations (Table I).

Methods

Fluid compartments and total body electrolytes were assessed by the methods as proposed by Moore and associates.[6] The procedure was as follows: Patients reported to the Body Composition Laboratory in the fasting state. Total body potassium (TBK) was determined by whole body counting of ^{40}K.[10] Body weight and height were recorded and the urinary bladder was emptied in order to begin a timed urine collection. Twenty-five ml of deuterium oxide and fifty microcuries of ^{24}Na were administered orally in saline for determinations of total body water (TBW) and total exchangeable sodium (Na_e) and the sodium space as a measure of extracellular water (ECW). Ten ml of T-1824 (Evans blue) was administered intravenously for determination of plasma volume (PV). Blood samples were drawn at 15, 30 and 45 minutes for colorimetric analysis of T-1824 concentration, and the volume of distribution was obtained by extrapolation back to zero time. Urine was collected at 4 and 6 hours and aliquots were analyzed by mass spectrometer for the ratio of heavy hydrogen to natural hydrogen; the urine collection was continued for a total of 24 hours to allow for equilibration and to account for urinary loss of sodium. Blood was obtained after 24 hours and a spot urine was collected during the following two hours for counting in a NaI well-type scintillation counter for gamma-emissions to determine specific activity. Stable sodium and potassium from serum and urine were analyzed by flame photometry. From the direct measurements of TBW, ECW, TBK, and serum potassium (SK) the following data were derived: intracellular water (ICW) = TBW — ECW; intracellular potassium concentration (ICK) = TBK — ECK (extracellular potassium)/ICW; body cell mass (BCM) = ICW/0.727 (assumed hydration); total body fat (TBF) was calculated according to the formula of Pace and Rathburn[11] which assumes that the hydration of fat-free body is 73.2 per cent. In states of water excess, correction was applied based on the observed ratio [ICW/TBW

Table I. Body Composition in Renal Failure: Clinical data

Case No.	Diagnosis	Age	Sex	Symptoms	Duration of Hypertension (yrs.)	Water and Electrolyte Disorder
1.	APH	59	M	Headache, fatigue, weight loss	0	None
2.	PA	52	F	Fatigue, weakness	11	Hypokalemia
3.	IN	53	F	Fatigue, weakness, polyuria	1	Sodium depletion
4.	G	19	M	Fatigue, anorexia, weight loss	0.1	Sodium excess
5.	G	47	F	Confusion, weakness, fatigue	2	Water excess

IN = Interstitial nephritis
APH = Accelerated primary hypertension
PA = Primary aldosteronism
G = Glomerulonephritis

and calculated ratio ECW (diseased)/ ECW (before illness)] in accordance with the nomogram of Moore and associates.[6]

Predicted values for body fluid compartments and total body electrolytes were calculated on the basis of observed TBW and an assumed normal distribution for age, sex and weight. Further comparisons were made with average normal values for persons of similar age, sex and weight in accordance with the prediction equations of Moore and associates.[6]

RESULTS

The laboratory and clinical observations and body composition data for five patients selected for detailed description are presented in Tables II through XI. Observations include examination and laboratory findings and antihypertensive therapy at the time of the initial and on subsequent examinations. Each of the five patients is presented in narrative summary in order to correlate clinical and laboratory data with patient management.

Primary hypertension with renal failure (Case 1)

A man, 59 years of age, consulted us on July 8, 1969, because of hypertension and renal failure. The family history revealed no known incidence of renal disease or hypertension. The past medical history revealed no evidence of previous renal or genitourinary problems; however, prior to the observation of elevated blood pressure in June, 1969, he had not consulted a physician for more than twenty-five years. Symptoms described included polyuria for one year, nocturnal and early morning headaches for six months and progressive fatigue and weight loss for three months. Investigation under the supervision of his local physician revealed proteinuria, microhematuria and cylindruria. The blood urea nitrogen (BUN) was 64 and the serum creatinine 5.6 mg per 100 ml and the creatinine clearance 15 ml/min. Urograms revealed both kidneys to be diminished in size. Treatment with thiazide diuretics, methyldopa and guanethidine resulted in poor blood pressure control with levels ranging from postural hypotension to 250 mm Hg systolic and 160 diastolic, with associated weakness, easy fatigue and weight loss.

On examination the weight was 137 pounds and the blood pressure 230 mm Hg systolic and 140 diastolic (Table II). Funduscopic examination revealed changes of group III hypertension (K.W.B.) with severe retinal arteriolar sclerosis, narrowing and focal constrictions, exudates and hemorrhages. The heart was moderately enlarged and a short, low pitched systolic bruit was noted over the abdominal aorta. Urograms revealed no evidence of obstructive uropathy or calyceal abnormality; however, there was poor concentration of contrast medium with reduced renal mass with a pole-to-pole renal diameter of 11 and 12 cm on the right and left respectively. Renal angiography revealed no evidence of renal artery stenosis. The heart was enlarged on chest roentgenograms and the electrocardiogram revealed evidence for left ventricular hypertrophy. Urinalysis revealed proteinuria, microhematuria and cylindruria.

The patient followed a diet which contained 40 mEq of sodium for 10 days, and body composition studies were accomplished on July 17, 1969. The patient then followed a diet with 20 mEq of sodium for four days to stress further his capability of conserving sodium in the urine; body composition studies were then repeated (Table III). Thereafter a dietary regimen was established which included 50 gm of protein, 40 mEq of sodium and 2,000 calories. Methyldopa was used in divided dosage totalling 0.5 to 1.5 grams per day for blood pressure control and allopurinol was used for control of hyperuricemia. Because of continued azotemia, further protein restriction to 25 gm was effected in September, 1969, and resulted in a prompt decrease in the blood urea and serum creatinine levels (Table II). In November, 1969, carbohydrate supplements were added to the diet and resulted in a marked general improvement in sense of well being and a

Table II. Body Composition in Renal Failure: Laboratory and clinical observations in a 59-year-old man with primary hypertension

Date	Weight (lbs.)	Blood Pressure (mm Hg)	Medication	Serum						Urine				Ccr., ml/min
				mg/100 ml				mEq/L		mEq /24 hrs		gms /24 hrs		
				Urea*	Creat.	PO_4	Uric Acid	Na	K	Na	K	Protein	Nitrogen	
7/8/69	137	230/140	M,H,G	141	5.35	3.7	9.3	146	5.4	98		3.0	8.16	18
7/18/69	135	210/120	M				9.1	140	5.5	36	50			
7/22/69	135	160/100	M							17	45			
9/2/69	136	170/94	M,A	158	6.2		7.2	138	5.2				4.46	
10/20/69	137	130/90	M,A	66	4.25									
11/24/69	140	160/100	M,A	66	5.4		7.0			29		0.7	3.34	
1/28/70	148	150/100	M,A	63	5.15	3.7		141	4.8	28			4.1	
2/27/70	149	170/110	M,A,F	53	4.9			136	4.7	73			4.0	
4/20/70	150	180/114	M,A							99			3.8	
4/24/70	154	150/90	M,A	57	5.0			138	4.8	37			4.0	13

A = Allopurinol H = Hydrochlorothiazide F = Furosemide
M = Methyldopa G = Guanethidine *Blood

Table III. Body Composition in Renal Failure: Observations in a 59-year-old man with primary hypertension

Feature	7-17-69			7-22-69		
	Observed	Predicted*	Average†	Observed	Predicted*	Average†
Height (Cm)	172			172		
Weight (Kg)	61.5			61.5		
TBW (L)	33.6	33.6	34.3	33.6	33.6	34.3
TBF (Kg)	15.5	15.5	14.6	15.5	15.5	14.6
PV (Ml)	3143	2650	2709			
ECW (L)	16.0	15.9	16.2	16.1	15.9	16.2
Na_e (mEq)	2516	2691	2746	2458	2691	2746
S_{Na} (mEq/L)	142		135-145	137		135-145
ICW (L)	17.6	17.7	18.1	17.5	17.8	18.1
TBK (mEq)	2990	2872	2932	2993	2872	2932
S_K (mEq/L)	5.5		4.0-5.0	5.7		4.0-5.0
ICK (mEq/L ICW)	163	158	158	165	158	158
BCM (Kg)	24.3	24.5	24.9	24.1	24.5	24.9

*Calculated on basis of observed TBW.
†Corrected for age, sex and weight

weight gain of 14 pounds over the following five months. Urinary nitrogen excretion and blood levels of urea, creatinine, phosphorus and electrolytes reflected excellent nutritional balance. Blood pressure control was satisfactory when sodium restriction was severe; however, the patient continued to experience blood pressure elevation when dietary sodium was increased as is reflected in the excretion data. Use of furosemide in doses of 20 to 40 mg two to three days weekly resulted in marked lability of blood pressure; however, return to severe restriction of dietary sodium resulted in prompt restoration of blood pressure control. Severe sodium restriction resulted in satisfactory blood pressure control without further sacrifice of renal reserve.

Hypertension with potassium depletion and renal failure (Case 2)

A woman, 52 years of age, consulted us on January 27, 1970, because of hypertension and kidney disease. The patient's father had experienced severe hypertension which had required medical therapy

for many years, and a brother had experienced recurrent episodes of renal lithiasis. The patient had experienced toxemia with her first pregnancy at 22 years of age with a subsequent normal blood pressure during the postpartum period. Persistent hypertension was first noted at 41 years of age in 1959. Symptoms had included easy fatigue with effort for 10 years and severe and progressive muscle weakness for three years. Previous investigation included urography, renal angiography, evaluation of serum electrolytes and renal function testing. In 1962 an atrophic right kidney, hypokalemia and increased excretion of aldosterone were noted. A right nephrectomy was performed in 1963 because of "atrophic pyelonephritis." Subsequent therapy with many antihypertensive agents, including guanethidine, had not resulted in satisfactory blood pressure control. Potassium replacement therapy had in part ameliorated the patient's "weakness." Loss of renal reserve, unsatisfactory blood pressure control and progressive muscular weakness caused the patient to seek consultation with us.

On examination the weight was 151 pounds and the blood pressure ranged from 165 mm Hg systolic and 95 diastolic while on guanethidine therapy, to 190 systolic and 120 diastolic seven days after terminating therapy. Funduscopic examination revealed moderately severe retinal arteriolar narrowing with focal constrictions and hypertensive sclerosis but no evidence of retinopathy. A short low pitched systolic bruit was noted over the abdominal aorta, and the patient was moderately obese.

Roentgenograms of the chest revealed cardiac enlargement and the electrocardiogram revealed evidence for left ventricular hypertrophy. Urinalysis revealed proteinuria and occasional hyaline casts. Detailed studies of blood and urine are reported in Table IV and body composition studies in Table V.

Total body potassium on January 30, 1970, was 1730 mEq. The patient was then placed on a diet containing 40 mEq of sodium and 90 mEq of potassium, and supplemental potassium was given orally at a dosage of 150 mEq per day for three days. Body composition studies were accomplished on February 3, 1970, while the patient continued on the described dietary regimen. Peripheral venous renin activity was determined on February 6, 1970, after the patient had been on a 20 mEq sodium and 90 mEq potassium dietary regimen with chlorothiazide, 0.5 gm twice daily for three days and after assuming the upright position for 4 hours prior to the study. Renin activity was not detectable in the peripheral venous blood by the Boucher technique.

On February 7, 1970, the patient was placed on a therapeutic regimen which included spironolactone, 100 mg daily, potassium supplementation orally at 100 mEq per day for 7 days, a diet limited to 20 mEq of sodium and 1,000 calories per

Table IV. Body Composition in Renal Failure: Laboratory and clinical observations

Date	Weight (lbs.)	Blood Pressure (mm Hg)	Medication	Serum						Urine				Cin., ml/min
				mg/100 ml				mEq/L		mEq/24 hrs		gms/24 hrs. Protein	mcg/24 hrs. Aldosterone	
				Urea*	Creat.	PO_4	Uric Acid	Na	K	Na	K			
1/28/70	151	165/95	G	48	2.10		8.4	146	1.6	90	40			
1/30/70		180/110	0		2.05			149	2.0	90	29	1.1	15	
2/4/70	151	180/114	0					150	2.1	49	36			45
2/6/70		190/120	0						2.1					
3/18/70	133	140/95	S	69	2.35			138	4.1	57	56		29	27
6/24/70	132	130/84	S	99	2.6	3.5	8.6	138	5.1	93	58			25

G = Guanethidine *Blood
S = Spironolactone

Table V. Body Composition in Renal Failure:
Observations in a 52-year-old woman with hypertension and potassium depletion

Feature	2-3-70			3-18-70			6-24-70		
	Observed	Predicted*	Average†	Observed	Predicted*	Average†	Observed	Predicted*	Average†
Height (Cm)	164			164			164		
Weight (Kg)	68.4			60.4			60.1		
TBW (L)	35.5	35.5	31.4	32.1	32.1	29.0	31.7	31.7	28.9
TBF (Kg)	24.6	24.6	25.5	19.2	19.2	20.8	17.3	17.3	20.6
PV (Ml)	3802	3228	2820	3428	2891	2580	2727	2846	2570
ECW (L)	19.1	17.1	15.2	15.5	15.5	14.0	15.7	15.3	14.0
Na_e (mEq)	3182	2754	2428	2374	2483	2235	2402	2448	2228
S_{Na} (mEq/L)	150		135-145	138		135-145	138		135-145
ICW (L)	16.4	18.4	16.2	16.6	16.6	15.0	16.0	16.4	14.9
TBK (mEq)	2021	2973	2628	2446	2689	2426	2625	2651	2418
S_K (mEq/L)	2.1		4.0-5.0	4.1		4.0-5.0	5.1		4.0-5.0
ICK (mEq/L ICW)	120	158	158	143	158	158	159	158	158
BCM (Kg)	22.6	25.3	22.3	22.9	22.9	20.6	22.0	22.5	20.6

*Calculated on basis of observed TBW
†Corrected for age, sex and weight

day and guanethidine, 5 to 25 mg per day as necessary to effect blood pressure control to 90 mm diastolic or less in the standing position. Symptomatic improvement was rapid, and the patient required no guanethidine for blood pressure control after two weeks. Body composition studies were again accomplished on March 18, 1970, and are recorded in Table V. The serum potassium and total body potassium had been restored to normal levels; however, in spite of elevated total body sodium and a significant elevation of plasma volume, the inulin clearance had diminished, signifying a significant decrease in glomerular filtration. Urinary aldosterone excretion was increased at 29 micrograms per 24 hours. The patient was continued on sodium and caloric restriction with spironolactone as necessary for blood pressure control, and additional studies were accomplished on June 24, 1970 (Tables IV and V), when the patient was normotensive and asymptomatic. Body composition was restored to near normal values, renal function was stable and dietary protein was adjusted to relieve nitrogen retention. Operation for suspected primary aldosteronism was deferred.

Hypertension with sodium-wasting renal disease (Case 3)

A woman, 53 years of age, consulted us on July 31, 1968, because of renal failure and hypertension. A sister was known to have systemic lupus erythematosus with nephritis and impaired renal function; however, there was no other known family history of renal disease or hypertension. Subtotal gastric resection had been accomplished in 1960 because of a gastric ulcer with two previous major bleeding episodes. Phenacetin-containing compounds had been employed for many years for recurrent headaches, and absorbable alkali for five or six years because of "ulcer" symptoms. Elevated blood pressure was first observed in 1967 and investigation at that time revealed severely impaired renal function with diminished renal size and scattered areas of nephrocalcinosis with poor medium concentration by urography. Anorexia, weight loss, easy fatigue and nocturnal polyuria had been noted for six months and hematinics and blood transfusions had been employed because of anemia. On examination, the blood pressure ranged from 140 to 170 mm Hg systolic and 90 to 120 diastolic. Funduscopic examination revealed retinal arteriolar narrowing with focal constrictions but no evidence of chronic hypertensive sclerosis nor retinopathy. Urinalysis revealed proteinuria, microhematuria, and cylindruria, and the kidneys measured 9.0 and 10.0 cm from pole to pole on the right and left respectively and contained scattered areas of nephrocal-

cinosis. There was a moderate hyperchloremic acidosis with a base deficit of 9 mEq per liter by Astrup technique and a normochromic-normocytic anemia. The blood glucose, serum calcium, coagulation studies, flocculation test for rheumatoid factor, L. E. clot test, Gram's stain and culture of the urine, serum protein electrophoresis and x-rays of the chest gave normal or negative results. Additional laboratory investigations are reported in Table VI.

A conservative program for management of the patient's renal failure and hypertension included a 30-gram protein, 40 mEq sodium dietary program with sufficient calories to maintain weight. The patient followed a program of approximately 60 grams of protein and 50 mEq of dietary sodium and continued to experience fatigue, blood pressure elevation, intermittent edema and anemia. Medical management by her family physician consisted of periodic transfusions and intermittent hydrochlorothiazide. Re-examination was accomplished on March 3, 1969, and revealed no significant change. The patient was re-instructed in a 30-gram protein renal failure dietary regimen with 40 mEq of sodium, and furosemide, 40 mg each Monday, Wednesday and Friday, was substituted for the intermittent hydrochlorothiazide. During the ensuing four months, the patient experienced progressive weight loss, fatigue, postural hypotension and severe weakness, and on re-examination on July 16, 1969, was found to be severely uremic, normotensive, but without overt clinical evidence of extracellular volume depletion. Laboratory observations are recorded in Table VI. The therapeutic regimen was altered to include 16 grams of protein, 40 mEq of dietary sodium, 2,500 calories which included carbohydrate supplements, sodium bicarbonate which included approximately 60 mEq of sodium per day, allopurinol 50 mg twice daily, vitamin supplements and continued use of furosemide in the previous dosage. The patient experienced rapid and profound symptomatic improvement complicated only by blood pressure elevation which required the addition of methyldopa in dosage of 250 to 750 mg daily for blood pressure control. Laboratory observations on subsequent examinations in August and November, 1969, and March, 1970, are recorded in Table VI, and body composition studies obtained after restoration of body water and sodium are recorded in Table VII. Careful regulation of dietary sodium, protein, calories, and medical antihypertensive therapy has resulted in restoration of normal body cell mass, water and electrolyte metabolism, blood pressure control, improved renal function and alleviation of need for transfusions.

Table VI. Body Composition in Renal Failure: Laboratory and clinical observations in a 53-year-old woman with hypertension and sodium-wasting renal disease

Date	Weight (lbs.)	Blood Pressure (mm Hg)	Medication	Serum						Urine				Ccr., ml/min
				mg/100 ml				mEq/L		mEq/24 hrs		gms/24 hrs		
				Urea*	Creat.	PO_4	Uric Acid	Na	K	Na	K	Protein	Nitrogen	
8/1/68	120	160/100	H	160	6.9	5.2	8.3	141	5.0	143	32	0.3		12
3/3/69	120	150/80	F	172	6.45	6.5	8.5	141	4.6	44				10
7/16/69	108	130/86	F,M	294	14.0	8.1	8.6	142	3.9	60			7.28	2
8/15/69	111	170/96	F,B,A,M	40	5.9	4.9	4.9	144	5.4	120	64		3.29	15
11/25/69	114	170/120	F,B,A,M	69	8.25	5.4	4.1	144	5.3	189	72			9
3/10/70	128	155/92	F,B,A,M	77	7.65	6.3	5.2	139	5.2	142			2.89	11

F = Furosemide
B = Sodium bicarbonate
A = Allopurinol
M = Methyldopa
H = Hydrochlorothiazide
* = Blood

Table VII. Body Composition in Renal Failure: 53-year-old woman with hypertension and compensated sodium-wasting renal disease

Feature	3-11-70		
	Observed	Predicted*	Average†
Height (Cm)	163		
Weight (Kg)	58.4		
TBW (L)	28.1	28.1	28.1
TBF (Kg)	20.0	20.0	20.0
PV (Ml)	2829	2488	2494
ECW (L)	14.8	13.6	13.6
Na_e (mEq)	2384	2173	2175
S_{Na} (mEq/L)	139		135-145
ICW (L)	13.3	14.5	14.5
TBK (mEq)	2181	2339	2175
S_K (mEq/L)	5.2		4.0-5.0
ICK (mEq/L ICW)	160	158	158
BCM (Kg)	18.3	19.9	19.9

*Calculated on basis of observed TBW
†Corrected for age, sex and weight

Renal failure, hypertension and sodium excess (Case 4)

A man, 19 years of age, consulted us on February 10, 1970, because of renal failure and hypertension. The patient's mother had experienced apparent acute glomerulonephritis during childhood; however, the family history was otherwise negative for hypertension and renal disease. The patient had experienced no significant previous illnesses and examination for participation in high school athletics was normal in the fall of 1966. A similar examination during the fall of 1967 revealed evidence of proteinuria and microhematuria on urinalysis. He experienced no symptoms until January, 1970, when an upper respiratory infection with associated persistent weakness, fatigue, anorexia and weight loss caused referral to our care. Previous investigation in 1967 had revealed a normal excretory urogram, serum creatinine, uric acid and BUN. In 1968 the serum creatinine was 1.2 mg per 100 ml and the creatinine clearance 82 ml per minute and the urinalysis revealed proteinuria, microhematuria and cylindruria. On examination the weight was 173 pounds and the blood pressure ranged from 170 to 190 mm Hg systolic and 110 to 140 diastolic. Funduscopic examination revealed severe retinal arteriolar narrowing and focal constrictions with hemorrhages, exudates, and papilledema but no evidence of chronic hypertensive sclerosis. The central venous pressure did not seem elevated and there was no evidence of peripheral edema. Roentgenograms of the chest revealed cardiac enlargement and there was evidence for left ventricular hypertrophy by electrocardiogram. Urinalysis revealed proteinuria, microhematuria, cylindruria, oval fat bodies and free lipids. Coagulation studies and examination of blood and urine for fibrinogen split products revealed equivocal findings. Percutaneous renal biopsy revealed changes consistent with a rapidly progressive proliferative glomerulonephritis. There was a normochromic-normocytic anemia and slight reduction of the serum albumin fraction. However, plasma lipids, serum calcium, blood glucose and the L. E. clot test gave normal or negative results. Additional laboratory and clinical observations are recorded in Table VIII, and body composition studies in Table IX.

Medical management included a diet containing 30 grams of protein and 40 mEq of sodium with calories as necessary for weight maintenance. Methyldopa was employed in doses of 250 to 750 mg per

Table VIII. Body Composition in Renal Failure: Laboratory and clinical observations in a 19-year-old man with hypertension and sodium excess

Date	Weight (lbs.)	Blood Pressure (mm Hg)	Medication	Serum						Urine				Cin., ml/min
				mg/100 ml				mEq/L		mEq/24 hrs		gms/24 hrs		
				Urea*	Creat.	PO_4	Uric Acid	Na	K	Na	K	Protein	Nitrogen	
2/11/70	173	170/130		132	5.55	7.8	8.6	141	5.0	148		6.9		
2/13/70	174	140/90	M							93	42		10.3	12
2/17/70	174	130/80	M							52				
2/23/70	174	150/95	M		5.5									13
2/25/70	174	160/100	M		5.35									
3/16/70	167	150/90	M							75			7.2	
4/9/70	164	150/90	M							81			7.8	
4/13/70	164	160/100	M	77	5.4	5.7	7.7	141	4.5	62			8.0	15

M = Methyldopa
*Blood

Table IX. Body Composition in Renal Failure: Observations in a 19-year-old man with hypertension and sodium excess

Feature	2-12-70			4-14-70		
	Observed	Predicted*	Average†	Observed	Predicted*	Average†
Height (Cm)	175			175		
Weight (Kg)	79.7			75.0		
TBW (L)	50.0	50.0	45.8	46.5	46.5	43.9
TBF (Kg)	11.5	11.5	17.1	11.4	11.4	15.0
PV (Ml)	4977	4059	3701	4263	3761	3539
ECW (L)	22.8	20.4	18.7	20.7	19.0	17.9
Na_e (mEq)	3449	3325	3043	3265	3090	2915
S_{Na} (mEq/L)	136		135-145	142		135-145
ICW (L)	27.1	29.6	27.1	25.8	27.6	26.0
TBK (mEq)	4434	4760	4365	4057	4431	4187
S_K (mEq/L)	4.0		4.0-5.0	4.6		4.0-5.0
ICK (mEq/L ICW)	160	158	158	153	158	158
BCM (Kg)	37.3	40.7	37.3	35.5	37.9	35.8

*Calculated on basis of observed TBW
†Corrected for age, sex and weight

day as required to maintain a standing diastolic blood pressure of 90 to 100 mm of mercury.

The patient experienced rapid symptomatic improvement and the blood pressure was readily controlled with methyldopa, 250 mg once or twice daily. Weight loss and hunger caused the patient to consume approximately 50 grams of protein per day with a sodium content of 60 to 80 mEq. Re-examination was accomplished on April 13, 1970. The weight was 164 pounds and the blood pressure ranged from 140 to 160 mm Hg systolic and 85 to 100 diastolic. Laboratory and clinical observations are recorded in Table VIII, and body composition studies in Table IX. The dietary regimen was revised to include 50 grams of protein, 40 mEq of sodium and approximately 2,500 calories with carbohydrate supplements. Careful regulation of dietary sodium, protein, calories, and medical antihypertensive therapy has resulted in blood pressure control and restoration of near normal water and electrolyte metabolism without significant further impairment of renal function.

Renal failure, hypertension, and water excess (Case 5)

A woman, 47 years of age, consulted us on November 24, 1969, because of renal failure and hypertension. Two sisters had experienced recurrent episodes of symptomatic lower urinary tract infection, and one brother was reported to have renal disease of unknown etiology. There was no known family history of hypertension. The patient had not known of any serious illness prior to August, 1962, when proteinuria and microhematuria were discovered at the time of a general physical examination. Elevated blood pressure was noted in 1968 and medical antihypertensive therapy was instituted. Easy fatigue, polyuria, weight loss and anemia were first noted in July, 1969, and became progressive thereafter. Previous investigation had included excretory urography in 1962 which revealed normal renal mass. Renal function had been on an intermittent basis from 1962 until the time of referral, and these observations revealed evidence of progressive loss of renal reserve. Progressive weakness, poor blood pressure control, weight loss from 135 to 120 pounds and renal failure caused referral to us for nephrologic evaluation.

On examination the weight was 117 pounds and the blood pressure ranged from 170 to 180 mm Hg systolic and 110 to 130 diastolic. Funduscopic examination revealed retinal arteriolar narrowing with focal constrictions, slight hypertensive sclerosis but no evidence of retinopathy. There was an azotemic odor, slight ankle edema and the patient appeared malnourished and chronically ill. Urograms revealed the renal silhouettes to be of normal size and configuration; however, there was poor concentration of contrast medium. Urinalysis revealed proteinuria, microhematuria, and cylindruria. There was a normochromic-normocytic anemia and a slight acidosis with a base deficit of 5 mEq per liter by the Astrup technique. The serum protein electrophoresis, Gram's stain and culture of the urine, plasma lipids, x-rays of the chest, L. E. clot test and electrocardiogram gave normal or negative results. Additional clinical and laboratory observations are recorded in Table X. The clinical observations strongly suggested chronic glomerulonephritis with renal failure and hypertension; accordingly, renal biopsy was deferred and a conservative program for medical management was developed. The dietary regimen included 30 grams of protein, 40 mEq of sodium, and calories as required for weight maintenance purposes. Methyldopa was recommended as required to maintain the standing diastolic blood pressure at 90 to 100 mm of mercury. Estimated liquid intake at the time of examination was 3,000 to 4,000 ml per day and diuretics had been employed because of edema. The patient was urged to restrict dietary liquids to those required to maintain a urine volume of approximately two quarts per day to avoid excess weight gain and edema.

The patient was able to accomplish the recommended protein and sodium restriction; however, profound thirst caused continued ingestion of large quantities of liquids and resulted in rapid weight gain with peripheral edema and hyponatremia (Table X). Chronic water excess persisted in spite of advised severe restriction of dietary liquids and sodium, and resulted in confusion, stupor and near coma. Hospitalization was accomplished under the supervision of the family physician on January 28, 1970. Fluids were restricted to 500 ml per day and the patient was given 500 mEq of sodium in the form of intravenous hypertonic (5 per cent) sodium chloride. Mental confusion worsened. The patient was transferred to our care on January 30, 1970, and severe restriction of dietary liquids and sodium was effected. The urine was virtually sodium-free (Table X); weight loss was slowly accomplished but there was little associated improvement in the patient's lethargy and confusion. Intravenous, and subsequently oral, diuretics were employed with more rapid weight loss and marked clinical improvement. Body composition studies were undertaken on February 11, 1970,

Table X. Body Composition in Renal Failure: Laboratory and clinical observations in a 47-year-old woman with hypertension and water excess

Date	Weight (lbs.)	Blood Pressure (mm Hg)	Medication	Serum						Urine				Ccr., ml/min
				mg/100 ml				mEq/L		mEq/24 hrs		gms/24 hrs		
				Urea*	Creat.	PO_4	Uric Acid	Na	K	Na	K	Protein	Nitrogen	
11/26/69	117	180/130	M,G,H	159	6.6	8.4	7.4	136	5.1	133	60	2.7	7.16	12
12/8/69	128	160/120	M	67	6.0			109	3.5	31	50			
12/12/69	125	150/100	M	78	5.45			115		28				
1/31/70	130	160/120	M	70	6.9			108		7				
2/3/70	122	150/94	M,F					119		150			6.68	
2/7/70	114	122/70	M,F					134						
2/10/70	113	140/90	M,F	107	6.75		9.2	137	3.9					
2/14/70	109	130/90	M,F							152	24			
2/17/70	108	140/90	M,F		5.8			139	4.9	93	38		6.0	
2/21/70	106	140/90	M							20	37			
2/24/70	107	140/95	M	70	5.95		9.0	138	4.6	10	38			13
2/27/70	107	150/100	M							10	33		4.52	

M = Methyldopa G = Guanethidine *Blood
F = Furosemide H = Hydrochlorothiazide

and revealed severe persistent excess in total body water with marked reduction of the intracellular potassium concentration in spite of normal or slightly increased total body potassium. Total body sodium was moderately increased in spite of the significant diuresis and saluresis recently effected. Continued restriction of dietary liquids and sodium resulted in additional weight loss and excellent blood pressure control; however, residual excess in the total body water and slight sodium excess remained, as determined by body composition studies (Table XI). The patient and her husband were again instructed in a diet containing 30 gm of protein, 20 mEq of sodium, fluid restriction and approximately 2,500 calories, including carbohydrate supplements, with the hope that she might more accurately follow the nutritional regimen and maintain blood pressure control, water, electrolyte and nitrogen balance and effect restoration of body cell mass. More careful observation of the outlined program has resulted in significant clinical improvement with slow but progressive weight gain, good blood pressure control and no overt edema.

Additional Patients

Similar clinical, laboratory and body composition studies were accomplished in 15 additional patients, 8 men and 7 women. All patients consulted us for examination because of renal failure and hypertension. Symptoms were those of renal failure, easy fatigue, weight loss, poor blood pressure control, anemia, and in some instances symptomatic uremia. Blood pressure ranged from 160 to 250 mm Hg systolic and 100 to 150 diastolic. Two patients with chronic primary hypertension had funduscopic evidence of chronic hypertensive sclerosis; however, the 13 patients with primary renal disease mostly had angiospastic retinal arteriolar changes which on occasion included retinopathy. Blood urea ranged from 100 to 310 and serum creatinine from 4.1 to 24.5 mg per 100 ml. The serum phosphate and uric acid determinations were elevated in all patients and the creatinine or inulin clearance was 20 ml per minute or less. Patients with polycystic renal disease, chronic recurrent bacteriuria with pyelonephritis and interstitial nephritis demonstrated little capacity to retain sodium and were without evidence of potassium excess unless severely oli-

Table XI. Body Composition in Renal Failure:
Observations in a 47-year-old woman with hypertension and water excess

Feature	2-11-70			2-26-70		
	Observed	Predicted*	Average†	Observed	Predicted*	Average†
Height (Cm)	167			167		
Weight (Kg)	50.8			48.5		
TBW (L)	35.4	35.4	26.0	33.1	33.1	25.2
TBF (Kg)	5.9	5.9	15.2	5.5	5.5	14.1
PV (Ml)	3139	3217	2288			
ECW (L)	15.7	16.9	12.4	15.0	15.8	12.0
Na_e (mEq)	2370	2721	1981	2298	2539	1916
S_{Na} (mEq/L)	141		135-145	138		135-145
ICW (L)	19.7	18.5	13.6	18.1	17.3	13.2
TBK (mEq)	2332	2989	2200	2359	2794	2126
S_K (mEq/L)	4.0		4.0-5.0	4.5		4.0-5.0
ICK (mEq/L ICW)	115	158	158	127	158	158
BCM (Kg)	27.1	25.4	18.7	24.9	23.8	18.1

*Calculated on basis of observed TBW
†Corrected for age, sex and weight

guric. These patients responded poorly to diuretics and sodium restriction but favorably to a carefully developed nutritional regimen. Dietary sodium requirements ranged from 40 mEq to more than 130 mEq per day. Indeed, one patient with chronic recurrent symptomatic bacteriuria and renal failure experienced postural hypotension and oliguria after treatment with thiazide diuretics. Initial sodium requirements exceeded 300 mEq per day. With careful management of dietary protein and after sodium repletion and nitrogen balance were restored, uremic symptoms cleared, sodium requirements were stabilized at 90 mEq per day and hypertension was readily controlled with small doses of methyldopa.

Patients with primary glomerular disease generally tolerated high levels of dietary sodium less well and often experienced severe blood pressure elevation under such circumstances. Indeed, on occasion it was necessary to restrict dietary sodium to 20 mEq per day and to use diuretics intermittently because of significant edema. Repeated 24-hour urine specimens, in the absence of diuretics, provided documentation of individual patient's capacity to conserve sodium with advanced renal failure. Regulation of dietary liquids commonly requires careful scrutiny in order to avoid edema in such instances. Blood pressure control with medical antihypertensive agents was far more readily achieved when sodium balance was accomplished at a level when the extracellular space was not markedly overexpanded. Similarly, maximum glomerular filtration, as determined by clearances of inulin or creatinine, was achieved only when the plasma volume was expanded beyond the mean normal range.

SUMMARY AND CONCLUSIONS

1. Twenty patients with renal failure and hypertension were submitted to extensive investigation of renal function, body composition and requirements of dietary protein, water and electrolytes.

2. Detailed descriptions of observations from the clinical history, physical examination and laboratory observations were presented from five cases selected to demonstrate commonly observed clinical problems in the management of patients with renal failure and hypertension.

3. Patients with primary hypertension or glomerulonephritis and hypertension with renal failure seldom tolerated excessive dietary sodium, water and protein without symptomatic uremia, severe blood pressure elevation and sodium and water retention. Careful control of dietary

sodium, water and protein resulted in profound symptomatic improvement and relatively easy control of hypertension with minimal medical antihypertensive therapy.

4. Severe sodium restriction or antihypertensive therapy with diuretic agents, or both, frequently resulted in depletion of body water and sodium, postural hypotension and oliguria with more severe uremia in patients with polycystic renal disease or interstitial nephritis and renal failure. Determination of sodium, water and protein requirements and carefully constructed dietary programs with a slightly-to-moderately expanded extracellular space resulted in improved renal function without seriously complicating medical management of hypertension by nondiuretic therapeutic agents.

5. Capability of sodium conservation by the hypertensive patient in renal failure is not uncommon. However, such patients can not rapidly adjust to sudden major changes in dietary water, electrolytes or protein, and satisfactory therapeutic programs might best be accomplished by study of requirements by metabolic balance and whole body composition methods over a period of weeks or months.

6. Our experience has indicated that poor control of dietary sodium and nitrogen will render satisfactory control of severe hypertension and renal failure most difficult. Further, patients with hypertensive renal disease may tolerate sufficient restriction of dietary sodium to permit better blood pressure management without experiencing depletion of body fluid compartments and sodium stores and further deterioration of renal function. Indeed, appropriate therapy for severe hypertension may result in preservation or even improvement of renal reserve.

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James C. Hunt, M.S., M.D., who presented this paper, is internationally known for his work on hypertension, particularly for his studies of hypertension associated with renal artery stenosis. Among his major interests are the applications of nutritional and metabolic techniques and the tools of nuclear science to research in hypertension and renal disease. Dr. Hunt is an officer in a number of scholarly societies. He is associate professor of medicine at the Mayo Graduate School of Medicine, University of Minnesota. He received an M.D. from Bowman Gray School of Medicine. Since 1954 he has been on the staff of the Mayo Clinic, Rochester, Minnesota, where he is a medical consultant and chairman of the Division of Nephrology. Ladislav P. Novak, Ph.D., of the Department of Clinical Pathology, and Ralph A. Nelson, Ph.D., M.D., of the Department of Physiology, were associated with Dr. Hunt in this study.

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First International Platelet Symposium

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FIRST INTERNATIONAL SYMPOSIUM ON THE BIOCHEMICAL AND PHYSIOLOGICAL FUNCTION OF PLATELETS

Introduction

This issue of Rush-Presbyterian-St. Luke's Medical Bulletin presents seventeen abstracts by European and American scientists working in the field of blood platelets. Until recently, the major interest in blood platelet research has been in its medical application related to the pathogenesis and treatment of hemorrhagic and thrombotic diseases. Conferences on the subject, for the most part, have had a clinical orientation and have not been directed to a consideration of fundamental platelet phenomena which are basic to understanding the role of platelets in health and disease.

The research of the past five years, however, has produced a great deal of experimental data on the structure and functioning of platelets at the molecular level. New and provocative concepts concerning primary platelet phenomena, such as adhesiveness and aggregation and their role in hemostasis and thrombus formation are emerging.

As a result, it seemed a most appropriate time to bring together investigators with diverse backgrounds in molecular and cell biology to assess the current status of our knowledge, to look for imaginative and productive areas for future research, and to encourage closer contact between European and American researchers. An International Symposium on the Biochemistry and Physiology of Blood Platelets was therefore arranged and was held October 15-17, 1970, at Rush-Presbyterian-St. Luke's Medical Center in Chicago. A distinguished group of scientists from this country and abroad were in attendance, and abstracts of their presentations appear on the following pages.

Max E. Rafelson, Jr.
Francois M. Booyse

Max E. Rafelson, Ph.D., Professor in the Department of Biochemistry and Associate Dean for Biological and Behavioral Sciences and Services

Francois M. Booyse, Assistant Professor in the Department of Biochemistry

PLATELET SYMPOSIUM

MORPHOLOGY OF MAMMALIAN PLATELET MEMBRANE SYSTEMS AND THEIR DERIVATION

O. Behnke
University of Copenhagen, Copenhagen, Denmark

Abstract. The triple-layered surface membrane of the mammalian platelet is continuous with a tortuous canalicular system in the platelet interior. This system, called the *surface-connected system* (SCS) should rightly be considered part of the surface membrane. The SCS may possibly act as a membrane reservoir.

The outer aspect of the platelet membrane and the SCS are coated with a 100 to 200 Å thick amorphous layer which is stained by various reagents in current use for the demonstration of mucosubstances at the fine structural level. Presumably the coat consists of an acid mucosubstance *and* adsorbed plasma proteins. The stainability of the coat is not affected by repeated washings, but is altered by proteolytic enzymes.

It is conceivable that the coat plays a major role in such important aspects of platelet physiology as recognition, adhesion and aggregation.

Another membrane system is the *dense tubular system* (DTS) which seems to be morphologically independent of the SCS. The DTS consists of narrow tubules containing a rather electron-dense material, the nature of which is unknown. One DTS tubule is located just beneath the marginal bundle of microtubules suggesting a functional relationship, but the significance of the DTS remains elusive.

The platelet membrane is a derivative of the megakaryocyte plasma membrane. During megakaryocyte differentiation, multiple invaginations appear which coalesce and form the demarcation membrane system. The system is coated with a mucosubstance showing the same stainability as the platelet membrane.

There is some uncertainty as to the origin of the DTS, but it is assumed that it originates from ergastoplasm and Golgi complex.

INFLUENCE OF VARIOUS COMPOUNDS AND SURFACES ON BLOOD PLATELETS AND PLATELET AGGREGATION STUDIED WITH THE SCANNING ELECTRON MICROSCOPE

TORSTEIN HOVIG
Oslo University Institute of Pathological Anatomy, Rikshospitalet, Oslo, Norway

Abstract. Scanning electron microscopy allows a three-dimensional picture of structures, and this microscope therefore represents a useful tool for studies on cell surface and shape alterations. At present the resolving power of the microscope is limited to about 200 Å, but even so, important information can be obtained.

Changes in shape of blood platelets seem to be an early reaction in response to various stimuli. The circulating platelets are disc-shaped and most of the platelets maintain this shape when kept at 37° C in citrated PRP. When citrated plasma is cooled in an ice-bath, however, the platelets become spheric, with numerous *buds* and some pseudopod-like protrusions. ADP and collagen cause shape alterations; the platelets become spheric and show marked pseudopod formation. If the platelet-rich plasma is stirred so that aggregation occurs, the pseudopods interlace and form a meshwork.

Intravenous administration of colchicin to rabbits and rats leads to rapid alterations of the circulating platelets which become spheric and show bud formation. By transmission electron microscopy it can be demonstrated that the circulating platelets lose their microtubules, similarly to that observed by *in vivo* incubation of platelets with colchicin. In spite of this fact, the platelets can form pseudopods *in vivo* and *in vitro*, and they are able to phagocytize polystyrene latex particles.

If blood platelets are exposed to particulate matter, such as can be obtained by infusions of lipid suspension, the platelets do not aggregate. They phagocytize the lipid, a process which leads to some morphological alterations of the platelets. There is only little pseudopod formation. Similar findings are made when using suspensions of polystyrene latex instead of lipid suspensions.

When blood is passed through a glass bead column, the platelets interact with the glass surface. The portion of the platelet facing the glass shows numerous pseudopods spreading out on the glass surface, whereas the rest of the platelet appears spheric. Few platelet aggregates appear in the column, and thus the glass bead column technique probably reflects the adhesiveness of the platelets to glass.

GLYCOPROTEINS OF THE HUMAN PLATELET MEMBRANE

G. A. Jamieson
The American National Red Cross Blood Research Laboratory, Bethesda, Maryland

Abstract. The outer membrane of human blood platelets is rich in carbohydrate and may be involved in a number of its immunological and physiological activities. A method has been developed for the hypotonic lysis of glycerol-loaded platelets followed by density gradient centrifugation which gives rise to two different types of membrane vesicle which differ in ultrastructure and in lipid content but are virtually identical in chemical and enzymatic analysis. These analyses indicate that the platelet membrane is analogous to the plasma membrane of other cells and provides biochemical confirmation of the suggestion (Behnke) that the demarcation membrane arises from the plasma membrane of the megakaryocyte.

Tryptic digestion of the purified membrane fractions leads to the isolation of two major glycopeptides identical with those isolated from intact platelets. One of these is a macroglycopeptide (M.W. 120,000) containing about 70% carbohydrate with approximately equal numbers of N- and O-glycosidic linkages with proline as its principal amino acid. This macroglycopeptide may be part of a thrombocyte specific antigen. The second glycopeptide (M.W. 5,000) does not contain O-glycosidic linkages and has aspartic as its principal amino acid. About 10^5 and 10^6 chains, respectively, of each glycopeptide are found on the platelet surface.

Two glycoproteins may be visualized in the membrane preparations by gel electrophoresis following solubilization in detergents, and partial purifaction has been effected by appropriate techniques.

These results provide some insight into the structural contribution of glycoproteins to the platelet plasma membrane and suggest that the macroglycopeptide extends from the surface of the platelet as a semi-flexible rod.

This work was supported, in part, by USPHS Grants GM-13057 and AI-09017

HUMAN PLATELET CONTRACTILE PROTEINS: PROPERTIES, LOCATION AND FUNCTION

Francois M. Booyse and Max E. Rafelson, Jr.
Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Abstract. Autoradiography, immunochemical localization and physicochemical analysis of the contractile proteins obtained from the surface and cytoplasm of human platelets suggest that at least two forms of contractile proteins exist in this cell.

Platelet myosin has been extensively characterized and used as a means of studying the differences between these two contractile proteins. Platelet myosin was obtained from purified contractile protein by dissociation with ATP, and the following physicochemical properties determined: sedimentation coefficient (s^0_{20}, w), 6.8 S; molecular weight, 574,900 g mole^{-1}; frictional ratio (f/f^0), 3.41; amino acid composition; approximate dimensions and topography: rod-like tail, 1200-1600Å long by 15-20Å wide attached to a globular head, 60-80 Å wide.

Treatment of intact platelets with high KCl concentrations yielded a protein fraction that could be characterized in part as contractile protein by its typical contractile protein properties (precipitation at low ionic strength, ATPase activity, ATP sensitivity, viscosity, amino acid analysis) and by precipitation with specific antiserum. Exposure of intact platelets to EDTA, pronase and trypsin also yielded an immunochemically identifiable contractile protein fraction. This surface-localized contractile protein constitutes only about 6 to 10% of the total contractile protein obtained from digitonized cells. Digitonin lysis and extraction of these pre-treated cells yields the remainder of the contractile protein (90%). A comparison of the relative viscosities, amino acid composition of myosin moieties, and turnover of these contractile proteins, indicates a distinct difference between these proteins. Only the surface-localized contractile protein appears to be synthesized. This surface protein also stains intensely with ruthenium red.

By using a soluble horseradish peroxidase-antibody labeling technique, contractile proteins have been localized in the platelet cytoplasm and membrane (in ultrathin sections) and on the surface of intact platelets. Conformational changes induced in the surface localized contractile protein by ADP and ATP will be considered in relation to the possible role of this protein in cellular adhesiveness.

HUMAN PLATELET PROTEINS

P. Ganguly
The American National Red Cross Blood Research Laboratory, Bethesda, Maryland

Abstract. Albumin has been isolated from human blood platelets, and a systematic comparative study between serum and platelet albumins carried out. Serum albumin and platelet albumin have similar properties at neutral pH but show distinct differences at acid pH values or in presence of 8 M urea. With suitable control experiments, these differences have been shown to be due to possible mild proteolysis of the platelet albumin molecule. Similarly, platelet fibrinogen, isolated in presence of suitable inhibitors, possesses physicochemical and coagulation properties similar to plasma fibrinogen. In the absence of inhibitors platelet fibrinogen breaks down into smaller fragments and shows marked differences from plasma fibrinogen. It is concluded that platelet albumin and fibrinogen are identical to plasma albumin and fibrinogen. These studies show the importance of inhibiting possible proteolysis in platelet protein studies.

A thrombin-sensitive beta-globulin has been isolated from human blood platelets by acrylamide gel electrophoresis followed by gel filtration. The protein produced a single precipitin line with anti-platelet serum or anti-factor XIII serum, and none with anti-whole serum or anti-fibrinogen serum. However, some of its properties, particularly its interaction characteristics with thrombin, are distinctly different from plasma fibrin-stabilizing factor.

Supported in part by National Institute of Health Grant HE-12520

PLATELET FUNCTION: A GUIDE TO PLATELET MEMBRANE STRUCTURE

J. R. O'Brien
Saint Mary's General Hospital, Portsmouth, England

Abstract. Four main functions will be considered:
Platelet aggregation: The membrane change resulting in platelet stickiness may on occasions involve intra-platelet mechanisms but some factors influencing aggregation act on the membrane and so may give structural information.

The release mechanism probably involves platelet granules but the released materials must finally pass through an intact membrane; thus, changes in membrane permeability may affect release.

Platelet factor 3 (PF 3) is derived from membrane phospholipid. It exists in three forms: hidden in the membrane; exposed on the surface; and free, as particulate material separate from the platelet.

Platelet factor 4 (PF 4): The molecules responsible for this activity are less precisely identified but this activity also exists in three forms—hidden, exposed on the membrane, and free in a soluble form.

Cocaine, a lipophilic membrane-bound molecule, makes the platelets swell; it thus affects permeability. It also exposes PF 3. *Osmotic damage* exposes PF 3 and PF 4 and causes swelling and release. *Aspirin*, and many other membrane stabilizing anti-inflammatory drugs, prevents the exposure of PF 4 and release, but has no effect on PF 3. These drugs may be acting on a glycoprotein.

All the mucopolysaccharide stains (MPS) studied influence platelet aggregation. Lanthanum salts, ruthenium red, toluidene blue, and brilliant cresyl blue and strong alcian blue all inhibit aggregation and, incidentally, all neutralize heparin in clotting tests. Alcian blue, uniquely, at lower concentrations itself causes aggregation and release. At still lower concentrations it markedly potentiates aggregation induced by ADP, etc. The membrane is stained by ruthenium only when the membrane has been altered in various ways. A study of the conditions when the membrane takes up these MPS stains will surely give a clue to the nature of stickiness and/or aggregability. It is suggested that membrane MPS plays a central part in platelet aggregation.

THE ORIGIN AND FUNCTION OF PLATELET DENSE BODIES

James G. White
University of Minnesota, Minneapolis, Minnesota

Abstract. Blood platelets utilize three basic mechanisms to effectuate hemostasis: adhesion (aggregation), contraction, and secretion. Adhesion and contraction are independent phenomena, but secretion is absolutely dependent on contraction and markedly facilitated by aggregation. Secretion involves the transfer of endogenous chemical constituents contained inside platelets to the surrounding plasma. Cell damage has little or nothing to do with the release reaction. Products of secretion include the adenine nucleotides, ATP and ADP, serotonin, catecholamines, hydrolytic enzymes, cations and platelet factors 3 and 4. Two types of organelles, dense bodies and granules, are specific repositories for nearly all the substances extruded by platelets. Granules develop before separation from megakaryocytes, but dense bodies are formed in circulating cells during uptake and concentration of serotonin. The dense bodies originate from granules, and transformation resembles labilization of lysosomes. Elements of the open channel system penetrating close to some granules and mucopolysaccharide in the organelles, may facilitate the energy dependent uptake and concentration of the amine. Preservation of dense bodies for study in the electron microscope was related to a reaction of serotonin with glutaraldehyde yielding a Schiff base which selectively precipitated osmic acid in the organelles. The osmophilia of dense bodies was considered specific cytochemical evidence for their content of serotonin, and the basis for the marked electron opacity. However, dense bodies are apparent in thin sections of platelets fixed in glutaraldehyde alone, osmic acid alone, and in whole mounts of unfixed, unstained platelets. Chemical treatment of aldehyde fixed platelets before exposure to osmic acid permits extraction of dense bodies or their conversion to concentric lamellar configurations resembling phospholipid micelles. The inherent opacity of dense bodies is due to a high concentration of cations, most likely calcium. The importance of dense bodies in platelet secretory physiology is supported by investigations of bleeding in the Hermanski-Pudlak syndrome. Hemorrhagic problems in these patients are due to defective platelet secretory function. Their platelets are structurally normal, except for nearly complete absence of dense bodies. The contents of granules are also released from platelets after the secretion of dense bodies. Their function appears primarily related to the bactericidal activity of platelets in inflammatory reactions.

INDUCTION OF PLATELET AGGREGATION BY IMMUNE COMPLEXES

E. F. Lüscher
Theodor Kocher Institute, University of Berne, Berne, Switzerland

Abstract. Washed human platelets aggregate and show release reaction upon the addition of antigen-antibody complexes, of aggregated gamma-globulin, or of gamma-globulin coated polystyrene particles. The effect of such complexes thus resembles those brought about by thrombin or collagen. Except for calcium ions no co-factors are required in this reaction. The only prerequisite is that the complex involved is capable of activating the complement system. Thus, neither IgA-globulin nor bovine IgG aggregated by chemical or heat treatment will interact with platelets, since both are inert towards complement.

It has been shown that the presence of free amino groups is essential for this type of reaction. The situation is therefore not unlike the one encountered with collagen where blocking of free amino groups also abolishes the effect on platelets. Attempts to localize the essential groups in human IgG are described, and possible implications of the mechanism involved for the understanding of aggregation in general are discussed.

ROLE OF CYCLIC AMP IN PLATELET AGGREGATION

EDWIN W. SALZMAN
Harvard Medical College, Beth Israel Hospital, Boston, Massachusetts

Abstract. The relation of cyclic 3′, 5′-AMP to platelet function has been studied by investigating the influence of this compound and of its N^6-2′-O-dibutyryl derivative on platelet aggregation and other aspects of platelet behavior and by studying the adenyl cyclase and phosphodiesterase activity of homogenized platelets and the cyclic AMP content of intact platelets.

Dibutyryl cyclic-AMP and cyclic AMP inhibited platelet aggregation induced by ADP, epinephrine, collagen, and thrombin. The platelet "release reaction" was also inhibited: specifically, there was inhibition of the induction of platelet factor 3 activity and of the release of labelled 5-hydroxytryptamine. Platelet swelling produced by ADP was not inhibited. The activity of dibutyryl cyclic AMP was associated with uptake of the compound by platelets.

Platelet cyclic AMP was increased by prostaglandin PGE_1 by stimulation of adenyl cyclase and by caffeine by inhibition of phosphodiesterase. Epinephrine reduced platelet cyclic AMP by inhibition of adenyl cyclase, and α-adrenergic effect. NaF increased adenyl cyclase of platelet homogenates but did not alter the cyclic AMP content of platelets. Adenyl cyclase activity was inhibited by collagen, 5′-hydroxytryptamine, and thrombin. ADP, dibutyryl cyclic AMP, and cyclic AMP did not alter adenyl cyclase activity.

These observations and others to be presented are consistent with the hypothesis that platelet aggregation is favored by a decrease in platelet cyclic AMP and inhibited by an increase in cyclic AMP.

OBSERVATIONS ON THE RAPID MORPHOLOGICAL REACTION OF PLATELETS TO ADENOSINE DIPHOSPHATE

G. V. R. Born
Medical Research Council Thrombosis Group, Department of Pharmacology, Royal College of Surgeons of England, London, England

Abstract. An optical method used for measuring platelet aggregation was adapted for measurements of the change in shape of platelets that rapidly follows the addition of the aggregating agent adenosine diphosphate (ADP). Measurements were made using dilute suspension of platelets with sufficient EDTA to prevent their aggregation. Measurements of the velocity and magnitude of the optical effects of the shape change were highly reproducible. Volumetric measurements showed that the shape change is not associated with an increase in mean platelet volume. The velocity of the shape change had a temperature coefficient of about 4.5. The velocity and magnitude of the shape change were not affected by pH between 5.8 and 9.2. The dependence of the velocity of the shape change on the ADP concentration was in accordance with Michaelis-Menton kinetics. The K_m was about $7.2 \times 10^{-7} M$. The velocity of the shape change was inhibited competitively by ATP, adenosine and 2-chloro-adenosine but not by AMP. When the inhibitors were added after the maximum of the shape change they caused a concentration-dependent diminution in the record of the change. The results suggest that the shape change is initiated by reaction of the agonist ADP with specific receptor sites on the platelet membrane which leads to energy-requiring changes in the structures responsible for maintaining the disc shape of normal platelets.

ADENINE NUCLEOTIDES AND PLATELET FUNCTION

H. Holmsen and H. J. Day
Institute for Thrombosis Research, Rikshospitalet, Oslo, Norway, and Department of Medicine, Temple University, Philadelphia, Pennsylvania

Abstract. Platelets cannot synthesize adenine necleotides (AN) *de novo*, but do so from some preformed purines, *i.e.*, adenine, adenosine, but *not* hypoxanthine and inosine. Platelet AN are compartmentalized: one pool participates in metabolism, and another, not participating, is stored in specific granules. A significant part of the metabolically active AN is bound to proteins, but the function of these AN is not yet understood. During energy-requiring platelet functions, such as the release reaction, a rapid net depletion of metabolically active AN occurs, and hypoxanthine accumulates extracellularly.

During the platelet release reaction, stored AN are rapidly extruded to the cell environment. ADP is slowly dephosphorylated in plasma. Extracellularly this nucleotide induces three platelet functions: reversible platelet aggregation, "refractory period," and release reaction. The rate of de-aggregation does not correlate with ADP disappearance, but is apparently due to increasing "refractoriness" towards the aggregating effect of ADP. In plasma, only small amounts of ATP are converted to ADP. ATP is mainly depyrophosphorylated to AMP which, in turn, is broken down to the aggregation inhibitor, adenosine. This nucleoside does not inhibit by competition with extracellular ADP, but seemingly inhibits as it is transported across the platelet membrane.

ASPECTS OF THE PLATELET RELEASE REACTION

H. James Day and Holm Holmsen
Temple University, Philadelphia, Pennsylvania

Abstract. Like secretory cells, blood platelets have the ability to excrete a number of materials exclusively stored in granules. This process, the blood platelet release reaction, is regarded to be important in hemostasis and thrombosis. Platelet release is induced by different chemically unrelated compounds, yet the final two steps in the reaction, extrusion and intracellular response, are similar.

The reaction can be subdivided into at least three steps: induction, intracellular transmission, and extrusion, in which the first step seems to be complex, dependent upon the inducer and membrane phenomena.

Inducers may be divided into two groups: (1) those releasing substances from dense granules, *i.e.*, adenine nucleotides, serotonin, PF_4 and possibly metal ions; and (2) those which cause release of the aforementioned materials plus the content of α-granules: acid hydrolases.

Characteristic of the first group of release inducers is that certain membrane inhibitors are able to block release completely. The same effect of inhibitors can be overcome by increasing the concentration of inducers from the second group. This apparent discrepancy may be explained by differences in threshold for induction leading to differential release phenomenon.

Groups of patients have been shown to have bleeding phenomena with no other abnormality than failure of their platelets to undergo release reaction. Results obtained so far point to an inherent lack of the granule-stored compounds rather than failure in the membrane induction step.

LIPID METABOLISM IN HUMAN PLATELETS

PHILIP W. MAJERUS and NANCY LEWIS
Washington University School of Medicine, Saint Louis, Missouri

Abstract. Only platelets among the formed elements of the blood have an active fatty-acid synthesizing system. Acetyl CoA Carboxylase (ACC), the rate-limiting enzyme in fatty-acid synthesis, is present in human platelets at levels comparable to those found in liver. The platelet enzyme seems identical to enzyme isolated from liver, both in physical properties and in kinetic behavior. Antibody to rat liver ACC cross-reacts with human platelet enzyme.

In studies utilizing glycerol incorporation into platelets, *de novo* synthesis of all the major phosphoglycerides was demonstrated. The pattern of incorporation of glycerol into phospholipid was markedly altered by thrombin (1U/ml). Most notably, formation of phosphatidyl serine was stimulated five-fold within two minutes of thrombin addition to platelets. This effect of thrombin requires proteolytic action. Trypsin also produced similar patterns of alteration of phospholipid synthesis in five-fold higher concentration than thrombin. Pre-incubation of trypsin with soya bean trypsin inhibitor abolished this effect on phospholipid synthesis. The proteolytic action of thrombin on platelet membranes has been studied using acrylamide gel electrophoresis in sodium dodecyl sulfate. These experiments have demonstrated one major protein band which is altered by thrombin treatment. The function of this protein is under study, and preliminary results indicate that it is not fibrinogen.

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ENERGY METABOLISM OF AGGREGATING PLATELETS

MANFRED STEINER
Brown University Institute for Life Science, Pawtucket, Rhode Island

Abstract. Utilization and breakdown of glucose and fatty acids by aggregating platelets was studied *in vitro.* The aggregating agents utilized in this study included ADP, epinephrine, collagen, thrombin, and polystyrene-latex particles. Glucose-C^{14}, differentially labeled, was used to determine the metabolic pathways of preferential utilization of this substrate during aggregation, release reaction and under normal conditions. Glucose consumption and total platelet glycolysis were increased with all the aggregating agents tested. Anaerobic glycolysis was moderately enhanced (30 to 90 percent) but citric acid cycle (CAC) activity showed a 6- to 10-fold stimulation with thrombin or latex particles, and a 1.5- to 3-fold increase with collagen, epinephrine and ADP. The HMP activity remained essentially unchanged in the presence of aggregating agents, although thrombin and latex particles caused some recycling through the shunt. There was glycogenolysis in platelets under control and experimental conditions, most marked during the first hour of incubation. Thrombin and particles increased glycogen breakdown by 30 percent above the control value and also enhanced the incorporation of glucose carbons into lipids and proteins (2- and 10-fold above the control values).

The utilization of fatty acids as energy source for platelets was examined with palmitate^{-14}C bound to albumin. Normal non-aggregated platelets oxidized 1 to 2 n moles palmitate/10^9 platelets per hour. Most of the aggregating agents other than thrombin caused either no, or only a small, increase in fatty acid breakdown compared to normal. With thrombin, a very marked decrease in fatty acid oxidation was noted.

ELASTOLYTIC PROTEASE IN BLOOD PLATELETS

B. Robert and L. Robert
Laboratoire de Biochimie du Tissu Conjonctif (Equipe de Recherche du C.N.R.S.), Paris, France

Abstract. These studies, performed in collaboration with Drs. Caen and Legrand (Laboratoire d'Hémostase, Institut des Maladies du Sang, Hôpital Saint Louis, Paris), were undertaken in order to explore the possible role of platelet enzymes in the degradation of arterial elastic tissue. The use of a very sensitive method, with ^{125}I- and ^{131}I-labeled elastin (Europ J Biochem 11:62, 1969) enabled us to demonstrate in human platelets an enzyme(s) attacking fibrous elastin. This enzyme is liberated from platelets during their aggregation and viscous metamorphosis produced by collagen, ADP, adrenalin, triton-X-100 or sonication. The release is accompanied by an activation of the enzyme: the total activity recovered in the supernate and the particulate fraction is much higher than in the controls and depends on the releasing agent (Nature, *in press*). A triton-X-100 extract was fractionated with $AmSO_4$ and gel-filtration. The catheptic activity could be separated from the elastolytic activity, and some of the characteristics of these enzymes could be determined. As the degradation of elastin is one of the earliest events in atherosclerosis, it seems possible that the release of this enzyme from platelets is involved in the atherosclerotic process.

HETEROGENEITY OF HUMAN PLATELETS. I. METABOLIC AND KINETIC EVIDENCE SUGGESTIVE OF YOUNG AND OLD PLATELETS

Simon Karpatkin
New York University Medical Center, New York, New York

Abstract. Human platelets have been separated into two extreme density populations by centrifugation in specific density media. A large-heavy platelet population with specific gravity > 1.042 and a light-small population with specific gravity < 1.032 were obtained, each representing approximately 15 to 20 percent of the total population volume. The average volume per platelet of the separated large-heavy and light-small platelet populations was 12 and 5 μ^3 respectively. When data were expressed per milliliter platelets or per gram wet weight, the large-heavy platelet population had a 3.4-fold greater glycogen content, 2.2-fold greater ATP content, 1.9-fold greater reduced glutathione content, 5.9-fold greater rate of glycogenolysis, 2.6-fold greater rate of glycolysis, 2.9-fold greater rate of protein synthesis, 1.7-fold greater rate of glycogenesis, and 1.4-fold greater rate of glyconeogenesis. The large-heavy platelet had a 2.5-fold greater resistance to osmotic shock as measured by release of ATP or ADP at low ionic strength. Significant differences were not obtained with respect to total lipid content or total lipid synthesis; or with respect to ATP or reduced glutathione stability following incubation for 60 minutes at 37°.

All glycolytic and related enzymes which modulate the conversion of glucose to glyceraldehyde-3-P have been measured in both platelet populations. Large-heavy platelets had approximately 2-fold greater activity for the enzymes: glycogen synthetase, fructose-1-6-diphosphatase, hexokinase, phosphoglucomutase, phosphohexoseisomerase, and phosphofructokinase. No significant differences were found for phosphorylase, glucose-6-P-dehydrogenase, 6-P-gluconic dehydrogenase, aldolase, and triose isomerase.

Se-75-selenomethilnine studies in two healthy human volunteer subjects indicate that large-heavy platelets are young platelets.

CONSTITUTIONAL AND ACQUIRED ABNORMALITIES OF PLATELET AGGREGATION

J. P. Caen, H. Vainer, and H. Lukasiewicz
Laboratoire d' Hémostase, Hôpital Saint Louis, Paris, France

Abstract. Constitutional and acquired hemorrhagic disorders or recurrent thrombotic diseases have been associated with various patterns of abnormal platelet aggregation.

The classic methods used in the study of platelet aggregation or release in citrated platelet-rich plasma and in washed platelets are of limited value, so one has to be very cautious in interpreting them.

Therefore, a study of correlating platelet and megacaryocyte morphology with some parameters of platelet biochemistry, populations, aggregation and release was done in various platelet disorders.

In the constitutional hemorrhagic diseases, thrombasthenia, thrombopathy due to abnormalities in release, or platelet dysfunction due to plasmatic anomalies were studied. In the group of acquired thrombopathies, several diseases, especially leukemia and myeloproliferative disorders were examined, as well as recurrent thromboembolic disease.

Normal subjects living at high altitudes (12,000 feet or more) rarely develop thromboembolic phenomena: a study of platelet aggregation was performed in Bolivia and Peru in order to see whether there are different aggregation patterns among the populations living at high altitudes.

In these subjects, ADT-induced aggregation is diminished or rapidly followed by disaggregation.

OBSERVATIONS ON THE CLINICAL DIAGNOSIS OF PARKINSONISM

HAROLD L. KLAWANS, JR.

The diagnosis of parkinsonism is entirely clinical, based upon what the examining physician sees and feels.[1] The cardinal manifestations of this process are well defined and relatively easy to recognize. No data are available as to the accuracy of this diagnosis when made by physicians without specialty training in neurology. Accuracy here would be based upon confirmation of the diagnosis by neurologists with special interest in parkinsonism. A comparison of the clinical diagnoses of non-specialists with those of neurologists will have value in revealing the accuracy with which parkinsonism is diagnosed in general medical practice.

METHODS

Records of all patients referred to the Parkinson Clinic of Presbyterian-St. Luke's Hospital between January 1, 1969 and October 21, 1969 were reviewed. This clinic sees patients only on referral by their own physician. Physicians are asked to submit a brief form about the patient, including the diagnosis and historical manifestations of the patient's parkinsonism. All patients had been diagnosed as having parkinsonism. All were felt by their referring physicians to have severe enough disease for treatment with L-dopa. All patients were convinced that they had parkinsonism, and all were anxious to receive experimental drugs for their disease.

Patients referred by a neurologist, or those under the care of a neurologist, or those previously diagnosed by such specialists were excluded from this study. This left a total of 121 consecutive patients. Diagnostic criteria used have been published elsewhere[1] and are summarized in Table I. The diagnosis of parkinsonism was made in patients in whom the clinical picture was dominated by rigidity, bradykinesia, rest tremor and loss of postural reflexes. If these findings were completely overshadowed by other neurologic signs, the diagnosis was not made. Three such patients were found (see below). The group of patients having "intention tremor only" and no manifestations of parkinsonism has been subdivided on the basis of familial history of similar manifestations. Patients having cerebellar system disease had intention tremor of the arms as well as ataxia.

RESULTS AND DISCUSSION

The diagnoses other than parkinsonism made by neurologists in the 121 consecutive cases are listed in Table II. Parkinsonism was not confirmed in 20 cases (16.5%). Three of these patients had mild cogwheel rigidity but their neurologic disabilities were related to other symptoms. In two, the clinical picture was dominated by cerebellar dysfunction. They also had bilateral pyramidal tract disease. The third patient had severe dementia with minimal rigid-

From the Department of Neurology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Harold L. Klawans, Jr., M.D., Assistant Attending Neurologist

TABLE I

CARDINAL PHYSICAL MANIFESTATIONS OF PARKINSONISM

1. **Bradykinesia**
   Slowness in initiating and carrying out voluntary movements. This includes decreased spontaneous movements and loss of associated movements
2. **Rigidity**
   Manifesting itself as increased resistance to passive movement which is present throughout the range of motion of a joint, and involves the agonists and antagonists equally
3. **Tremor**
   Tremor must be present at rest and should be decreased or disappear on the initiation of voluntary movement
4. **Abnormal postural reflexes**
   This is clinically measured by observing the patient's response to sternal pressure

ity. The diagnosis of parkinsonism on referral was made on the basis of intention tremors in 14 instances, thus accounting for 70% (14 out of 20) of the incorrect diagnoses. None of these patients had any resting tremor or other manifestations of parkinsonism. Six had diffuse cerebellar system involvement with significant gait ataxia.

Patients referred by specialists in internal medicine totaled 41. The referring diagnoses were incorrect in six of these (14.3%). Other non-neurologists referred 80 patients, of whom 14 were incorrectly diagnosed (17.5%).

From this it appears that the clinical diagnosis of parkinsonism by non-neurologists is incorrect in approximately one out of six patients. It should be emphasized that these were not patients with early manifestation, but all had well-established neurologic disease with significant disability. This is especially important because all patients were referred for L-dopa therapy. While effective in parkinsonism, the drug frequently produces significant although reversible toxic effects.[2,3] It is therefore reasonable to expect that had L-dopa been administered to all these patients, those without parkinsonism would have experienced only the toxic and not the beneficial effects of the drug.

The rather high percentage of incorrect diagnoses of this common condition is unfortunate. It could perhaps be low-

TABLE II

NEUROLOGIC DIAGNOSIS IN 121 PATIENTS REFERRED WITH PARKINSONISM

		Number of Cases
Intention tremor only		8
a. With family history	3	
b. Without family history	5	
Cerebellar system disease only		6
Cerebellar system disease with minimal rigidity		2
Motor neuron disease		2
Dementia with minimal rigidity		1
Cervical osteoarthritis		1
Total incorrect diagnoses		20 (16.5%)

ered by further emphasis, in medical training and in the non-specialist literature, on correct diagnostic criteria for parkinsonism.

SUMMARY

Case records of 121 consecutive patients referred by non-neurologists for L-dopa treatment for parkinsonism were reviewed. Twenty patients (16.5%) did not have parkinsonism, and of these 20, intention tremor accounted for the misdiagnosis in 14. A correct neurologic diagnosis is essential so that the specific effectiveness of the drug is properly directed, without exposing patients without parkinsonism to its toxic effects.

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ANTIMICROBIALS, INFECTIONS, AND THE NURSE-EPIDEMIOLOGIST AT PRESBYTERIAN-ST. LUKE'S HOSPITAL

Lawerence D. Edwards
Rolando C. Balagtas
Ruth Lawrence
Lois Eisner
Paula Lowe
Stuart Levin
Mark H. Lepper

INTRODUCTION

Prevention of hospital-acquired infections presupposes knowledge of how hospital infections are acquired and that they are, indeed, preventable. Even more basic than this, one must be convinced that a significant portion of patients actually acquire infections in the hospital. In an effort to show that hospital-acquired infections occur in a significant percentage of patients, several authors have reported their experience with various infection surveillance systems.

A few authors have reported surveillance of only hospital-acquired infections.[1,2,3] Others have reported surveillance of both hospital-acquired and community-acquired infections associated with antimicrobial use.[4,5,6]

The authors of the present report believe that all hospitalized patients with any type of infection should be included in the surveillance program, for two major reasons. First of all, a true community-acquired infection in a hospitalized patient introduces an organism into the hospital which may spread to other susceptible hospitalized patients. Such a relationship may not be recognized without total surveillance. Secondly, in the light of present knowledge, it is difficult to delineate the "true" hospital-acquired infection. This is pointed out in the study by Kessner and Lepper on gram negative bacilli in the hospital and community.[5] Ninety-four percent of infections were of indeterminate classification when rigid classification criteria were used, *i.e.*, *community-acquired*: no hospital contact or antimicrobial therapy for two months; *hospital-acquired*: onset in the hospital and related to a hospital procedure and no antimicrobial therapy for two months.

From the Section of Infectious Diseases, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Lawerence D. Edwards, M.D., U.S.P.H.S. fellow in the Section of Infectious Diseases
Rolando C. Balagtas, M.D., Adjunct in Pediatrics
Ruth Lawrence, M.D., resident in Medicine
Lois Eisner, R.N., Section of Infectious Diseases
Paula Lowe, R.N., Section of Infectious Diseases
Stuart Levin, M.D., Assistant Attending Physician and Director of the Section of Infectious Diseases
Mark H. Lepper, M.D., Attending Physician and Consultant to the Section of Infectious Diseases

Practically, then, it becomes necessary to use the criterion of *time*, and to show this different criterion, we will substitute the word "onset" for "acquired." Therefore, *community onset* infections are those present or incubating on admission, and *hospital onset* infections are all those occurring after hospital admission.[6] The term "hospital associated infection" as defined by Brachman will be used in this report to include both community onset and hospital onset infections.[7] Hopefully, examination of data stratified by the criterion of time may yield epidemiologic clues and provide background data for more detailed studies of specific organism-acquisition rates among patients. Studies of the latter type may definitively separate the true community-acquired from the true hospital-acquired infection.

Since antimicrobials are an important factor in selecting prevailing strains of organisms,[6,8,9] they should be surveyed as well as all infections.

One report based surveillance on a review of bacteriology reports and subsequent chart reviews[2] but this type of surveillance is inadequate since up to 40% of infections in hospitalized patients are not cultured.[7] Some authors have suggested that the most adequate surveillance is obtained through utilization of the nurse epidemiologist.[1,10] During the past three years, this hospital has utilized two nurse epidemiologists who tabulated weekly all infections of hospitalized patients on the medical and surgical services. Services such as psychiatry, pediatrics, and obstetrics were not surveyed nor was there an attempt to survey antimicrobial use because of limited personnel.

Standardized criteria were used in diagnosing specific infections (Table I). Hospital charts often do not contain data sufficient to fulfill these standardized criteria. This system, therefore, requires nurses to make diagnoses on the basis of available data contained in the charts.

This study attempted to validate the accuracy of surveillance data obtained

TABLE I

CRITERIA USED FOR INFECTIONS

A. General criteria
   a. Gastro-intestinal Tract (Diarrhea—one of the following)
      1. Three stools a day for two days
      2. Watery or purulent stools
      3. Identification of pathogenic organisms such as Salmonella, Shigella, Staphylococcus aureus, and specific enteropathogenic serotypes E. coli
   b. Genital Tract
      1. Post partum fever (both of the following)
         a. Temperature 101° for 24 hours or longer
         b. Uterine tenderness
      2. Pelvic inflammatory disease
         Must carry actual stated diagnosis of physician making the examination
   c. Respiratory Tract (Lower Respiratory Tract—three or more of the following should be present)
      1. Cough
      2. Rales in lungs and/or dullness to percussion
      3. Purulent sputum
      4. Temperature 101° or greater
      5. Isolation from sputum of any of the pathogenic organisms: Diplococcus pneumoniae, Beta hemolytic streptococci, Klebsiella, E. coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus.
   d. Soft tissue (wounds and lesions)
      1. This includes boils, furuncles, carbuncles, stitch abscesses, and wound dehiscence
      2. Signs and symptoms are redness, heat and purulent drainage from any wound
   e. Urinary Tract Infections—At least two of the following should be present
      1. Significant bacteruria (10^5 or more bacteria per milliliter) in properly collected specimens
      2. Fever, chills, costovertebral tenderness
      3. Pyuria (5 WBC's or more per microscopic high power field of centrifuged urine specimen)

by two nurse epidemiologists under these conditions, and to compare data obtained independently by three physicians from the infectious disease section. Infection rates and antimicrobial use at this hospital were also determined.

METHODS

A 12-day survey was conducted from July 22 through August 2, 1968 in Presbyterian-St. Luke's Hospital, an 840-bed, university affiliated and teaching hospital, with an average patient stay of 12 days. During the survey period the two nurse epidemiologists continued their existing methods of surveillance which included daily review of all bacteriology reports in the laboratory, review of death certificates, and a weekly review of charts on all units except psychiatry, pediatrics, and obstetrics. Charts were selectively reviewed on the basis of clues for infections obtained from a perusal of the medication index and the procedure index for each patient.

Three physicians from the infectious disease section surveyed the same units, plus pediatrics and obstetrics, during this 12-day period. Their methods of surveillance entailed a thorough screening of admission charts, a chart review of all patients receiving antimicrobials, and a daily review of discharge charts in the medical records department. On seven medical and surgical units, systemic antimicrobial use was thoroughly reviewed for all patients. Each unit was visited two or three times per week.

Community onset infections were so designated if they were present on admission or became clinically manifest within the first 72 hours of hospitalization and were not related to a hospital procedure (surgery, catheterization, etc.). All infections suspected of being related to such a hospital procedure, or those clinically manifest after 72 hours were designated as hospital onset infections.[6] This was a period-prevalence study.

RESULTS

Comparison of data collected by the nurses with that obtained by the physicians on the medical and surgical services revealed a match of 152 patients. However, the physicians tabulated 202 infections, while the nurses tabulated 191 infections in these same 152 patients (Table II). Two additional discrepancies were evident: (A) Among the lower res-

TABLE II

COMPARISON OF 152 PATIENTS MATCHED BY THE PHYSICIANS AND NURSES BY INFECTION SITE AND HOSPITAL SERVICE

Anatomical Site of Infection	Service								Totals	
	Medicine				Surgery					
	Community*		Hospital*		Community		Hospital			
	MD	RN	MD	RN	MD	RN	MD	RN	MD	RN
Genitourinary	10	26	14	6	24	39	27	5	75	76
Lower Respiratory	18	21	10	5	9	11	21	11	58	48
Cutaneous	2	2	1	2	12	11	24	19	39	34
Intestinal	4	4	0	0	7	8	0	0	11	12
Bacteremia	3	4	0	0	1	0	5	5	9	9
Cardiovascular	1	2	1	0	0	0	2	0	4	2
Eye, Ear, Nose & Throat	1	2	1	0	0	0	0	1	2	3
Meningoencephalitis	3	2	0	0	0	0	0	0	3	2
Miscellaneous	0	3	0	0	0	2	1	0	1	5
Total	42	66	27	13	53	71	80	41	202	191

*Community onset infections were so designated if they were present on admission or became clinically manifest within the first 72 hours of hospitalization and were not related to a hospital procedure; hospital onset infections were so designated for all infections related to a hospital procedure or those clinically manifest after 72 hours of hospitalization. The same definitions apply to all tables.

piratory infections, the nurses found only 4 cases of bronchitis while the physicians found 15 cases. (B) While the overall number of genitourinary infections matched, there was disagreement in place of onset of the infection; the nurses favored community onset while the physicians favored hospital onset. These particular problems have been rectified by further nurse education.

Nurses classified 36 patients with 43 infections, but in the physicians' judgment there were insufficient data to warrant such classification. The physicians would have classified 20 of these patients under use of prophylactic antimicrobials without infections. The remainder were patients with urinary tract infections on whom there was indecision as to what colony count to use; the nurses used 10^4 colonies/cc, and the physicians required 10^5 colonies/cc. The nurses recognized 11 patients with 12 infections that the physicians did not find (Table III). Seven of these were cutaneous infections and five were urinary tract infections; these 11 patients were not on antimicrobials (see discussion). The physicians found 57 patients with 60 infections which the nurses did not find. The general distribution of these 60 infections was similar to the general distribution of all infections (Tables II and III).

Data obtained by the physicians on the medical and surgical services revealed an overall infection rate of 37.2% (Table IV). It is noteworthy that the hospital onset infection rate on the surgical service (26%) was over twice that on the medical service (11%), while the community onset infection rate was nearly identical on the two services.

Genitourinary (GU), lower respiratory infections (LRI) and cutaneous infections were the predominant infection sites on both medical and surgical services (Table V). Ninety-three percent of the GU infections were urinary tract infections; 55% of the LRI were pneumonias, 25% were bronchitic episodes, and 12% were pulmonary tuberculous infections; 55% of the cutaneous infections were surgical wound infections.

Forty-nine patients (23.4% of infected patients) on the medical and surgical services did not have cultures taken when indicated. Eighty percent of these patients were hospitalized for surgery, and 51% were surgical hospital onset infections (Table VI).

A total of 112 patients on all services had cultures yielding 267 significant isolates. The predominance of gram negative organisms is readily apparent upon examination of Table VII.

Twenty-five percent of 144 pediatric patients and 8.0% of 151 obstetric patients had an infection during their hospital stay (Tables VIII and IX). There were 124 newborn discharges with one infection noted—a bacteremic episode in an infant who received an exchange infusion.

Our hospital-associated infection rate

TABLE III

SURVEILLANCE DISCREPANCIES BETWEEN NURSES AND PHYSICIANS

Site of Infection	Number of Infections
A. Fifty-seven patients with sixty infections found by the physicians only	
Genitourinary	24
Lower Respiratory	16
Cutaneous	12
Intestinal	3
Bacteremia	1
Cardiovascular	1
Eye, Ear, Nose & Throat	2
Meningoencephalitis	1
B. Eleven patients with twelve infections found by the nurses only	
Cutaneous	7
Urinary	5

TABLE IV

COMMUNITY AND HOSPITAL ONSET INFECTIONS ON THE MEDICAL AND SURGICAL SERVICES

Classification	MEDICINE			SURGERY			TOTAL		
	Number of Infections	Number of Infected Patients	Percent Infected Patients*	Number of Infections	Number of Infected Patients	Percent Infected Patients*	Number of Infections	Number of Infected Patients	Percent Infected Patients*
Community onset	54	48	18.6	70	57	17.1	124	105	17.8
Hospital onset	30	25	9.7	97	81	24.3	127	106	17.9
Both Community Hospital onset	8	4	1.6	15	5	1.5	23	9	1.5
Total	92	77	29.9	182	143	42.9	274	220	37.2

*The population at risk on medicine was 258 patients and the population at risk on surgery was 333 patients with a total of 591 patients. The percentages of infected patients were calculated from these population bases.

TABLE V

DISTRIBUTION OF INFECTIONS BY SITE AMONG 220 MEDICAL AND SURGICAL PATIENTS SURVEYED BY PHYSICIANS

Site of Infection	Service				Total	
	Medicine		Surgery			
	Community	Hospital	Community	Hospital	Community	Hospital
Genitourinary	13	17	34	40	47	57
Lower Respiratory	22	13	11	27	33	40
Cutaneous	6	1	20	31	26	32
Intestinal	4	1	9	0	13	1
Bacteremia	4	0	1	5	5	5
Cardiovascular	2	1	0	2	2	3
Eye, Ear, Nose & Throat	2	1	1	0	3	1
Meningoencephalitis	4	0	0	0	4	0
Miscellaneous	1	0	0	1	1	1
Total	58	34	76	106	134	140

TABLE VI

DISTRIBUTION OF FORTY-NINE PATIENTS BY SITE AND SERVICE ON WHOM CULTURES WERE NOT DONE WHEN INDICATED

Site of Infection	Service			
	Medicine		Surgery	
	Community	Hospital	Community	Hospital
Genitourinary	1	0	6	6
Lower Respiratory	2	2	1	11
Cutaneous	2	1	2	8
Intestinal	1	0	4	0
Cardiovascular	0	1	0	0
Eye, Ear, Nose & Throat	0	0	1	0
Totals	6	4	14	25

TABLE VII

SIGNIFICANT BACTERIAL ISOLATES BY SITE OF INFECTION AND PLACE OF ONSET

ORGANISM	SITE															
	Genito-urinary		Lower Respiratory		Cutaneous		Intestinal		Bacteremia		Cardio-vascular		Other		Total	
	Comm	Hosp	Comm	Hosp	Comm	Hosp	Comm	Hosp	Comm	Hosp	Comm	Hosp	Comm	Hosp	Comm	Hosp
Escherichia Coli	18	21	1	4	5	4	1	0	2	2	0	0	0	0	27	31
Klebsiella-Enterobacter	5	19	3	12	6	9	1	0	0	1	0	0	1	0	16	41
Proteus Mirabilis	7	8	1	6	2	4	0	0	1	0	0	0	0	0	11	18
Pseudomonas	2	10	1	3	4	2	0	0	1	0	0	0	0	0	8	15
Streptococcus fecalis	2	10	0	0	1	6	0	0	1	0	0	0	0	0	4	16
Staphylococcus coag. pos.	1	0	0	1	4	4	0	0	2	0	0	0	0	1	7	6
Hemophilus Influenza	0	0	11	1	0	0	0	0	0	0	0	0	0	0	11	1
Staphylococcus coag. neg.	0	0	0	0	3	2	0	0	0	2	1	1	1	0	5	5
Serratia	0	7	0	2	0	1	0	0	0	0	0	0	0	0	0	10
Diplococcus pneumoniae	0	0	6	0	0	0	0	0	0	0	0	0	1	0	7	0
Grp. A-Beta Hemol. Strep.	0	0	1	0	2	0	0	0	0	0	0	0	0	0	3	0
Non Grp. A-Beta Hemol. Strep.	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	2
Citrobacter	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	2
Proteus vulgaris	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	3
Mycobacterium Tuberculosis	1	0	2	0	0	0	0	0	0	0	0	0	0	0	3	0
Herella	1	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0
Paracolon	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Other	1	0	1	0	1	0	2	0	0	0	1	0	2	1	8	1
Total	39	79	28	31	29	35	4	0	7	5	2	1	5	2	114	153

TABLE VIII

DISTRIBUTION OF INFECTIONS BY SITE AND PLACE OF ONSET AMONG THIRTY-EIGHT HOSPITALIZED PEDIATRIC PATIENTS

Site of Infection	Community Onset	Hospital Onset
Genitourinary	9	5
Lower Respiratory	8	2
Cutaneous	3	2
Intestinal	5	0
Eye, Ear, Nose & Throat	2	1
Meningoencephalitis	1	0
Totals	28	10

TABLE IX

DISTRIBUTION OF INFECTIONS BY SITE AND PLACE OF ONSET AMONG TWELVE HOSPITALIZED OBSTETRIC PATIENTS

Site of Infection	Community Onset	Hospital Onset
Genitourinary	7	2
Lower Respiratory	1	1
Cutaneous	0	1
Totals	8	4

on all services was 28.9% of patients; 12.9% had hospital onset, and 16.9% had community onset infections (Table X).

Three-quarters of all systemic antimicrobials used on the medical and surgical services were distributed among the first eight antimicrobials listed in Table XI. Ampicillin led the list with 21% of antimicrobial courses given. It is significant that 48% of all antimicrobials used were for "prophylactic" purposes (Tables XII and XIII). An example of therapeutic sequential use of systemic antimicrobials (Table XIII) was in a patient who received penicillin followed by methicillin followed by oxacillin-nalidixic acid in combination followed by ampicillin followed by cephalothin-kanamycin in

TABLE X

PERCENT OF PATIENTS WITH HOSPITAL ASSOCIATED INFECTIONS BY SERVICE AND PLACE OF ONSET

Place of Onset	Service					Total Hospital
	Medicine	Surgery	Obstetrics	Pediatrics	Newborn	
Community	20.2	18.6	5.3	19.4	NA*	16.9
Hospital	11.3	25.8	2.7	6.9	0.8	12.9

*Not applicable, since newborns cannot enter with a community infection. Thus, the total risk population for hospital onset infections is 1010 patients (258—medicine; 333—surgery; 151—obstetrics; 144—pediatrics; and 124—newborns) and the total risk population for community onset infections is 886 patients (excludes newborn).

TABLE XI

AVERAGE NUMBER OF SYSTEMIC ANTIMICROBIAL COURSES PER PATIENT ON MEDICINE AND SURGERY*

Antimicrobial	Service		
	Medicine	Surgery	Medicine & Surgery
1. Ampicillin	0.15	0.24	0.20
2. Penicillin	0.08	0.13	0.11
3. Short-acting Sulfa	0.05	0.12	0.08
4. Tetracycline	0.08	0.08	0.08
5. Cephalothin	0.08	0.08	0.08
6. Non-absorbable Sulfa	0.02	0.13	0.08
7. Cephaloridine	0.03	0.08	0.05
8. Streptomycin	0.02	0.08	0.05
9. Nalidixic Acid	0.06	0.02	0.04
10. Nitrofurantoin	0.05	0.03	0.04
11. Other Penicillinase-Resistant Penicillins	0.02	0.03	0.03
12. Kanamycin	0.02	0.03	0.02
13. Isoniazid	0.02	0.02	0.02
14. Neomycin	0.00	0.03	0.02
15. Chloramphenicol	0.00	0.03	0.01
16. Erythromycin	0.02	0.01	0.01
17. Lincomycin	0.00	0.03	0.01
18. Methanamine Mandelate	0.01	0.01	0.01
19. Other	0.01	0.05	0.03
Totals	0.72	1.23	0.97

*Based on a sample of 146 medical patients and 145 surgical patients making a total of 291 patients.

TABLE XII

PERCENT OF PATIENTS RECEIVING SYSTEMIC, PROPHYLACTIC ANTIMICROBIALS ON MEDICINE AND SURGERY

Antimicrobial	Service		
	Medicine	Surgery	Medicine & Surgery
Prophylactic—single	15.1	24.9	20.6
Prophylactic—combination	0.8	2.7	1.9
Prophylactic—sequential	1.9	8.8	5.8
Totals	17.8	36.4	28.3

Calculated as the number of patients receiving systemic antimicrobials per patient population at risk of 258 medical patients, 333 surgical patients or a total of 591 patients.

TABLE XIII

PERCENT OF PATIENTS RECEIVING SYSTEMIC, THERAPEUTIC ANTIMICROBIALS ON MEDICINE AND SURGERY

Antimicrobial	Service		
	Medicine	Surgery	Total
Therapeutic—single	13.6	18.0	16.1
Therapeutic—combination	2.7	3.6	3.2
Therapeutic—sequential	8.1	14.4	11.7
Totals	24.4	36.0	31.0

Calculated as the number of patients receiving systemic antimicrobials per patient population at risk of 258 medical patients, 333 surgical patients or a total of 591 patients.

combination followed by cephalothin followed by cephaloridine. While this patient would have been counted once in Table XIII (therapeutic sequential), he would have been counted eight times in Table XI, since he received eight different antimicrobials.

DISCUSSION

It is difficult to compare accurately infection surveillance data taken from several hospitals because of differences in patient population, methods of surveillance, hospital procedures, time of year the data was obtained, and the use of antimicrobials. While recognizing the many variables involved, there is value in scrutinizing data obtained by those who have wrestled with the problem. Some reports are prevalence studies,[3,4] while others are incidence studies of varying periods of time.[1,2,5,6]

Barrett, *et al.*,[4] reported a community "acquired" infection rate of 26.3% and a hospital "acquired" infection rate of 15.5% at Boston City Hospital. This was a prevalence study, and accordingly the rates were higher than incidence rates as discussed by Thoburn, *et al.*[1] The latter [authors], in a six-month incidence study at John Hopkins Hospital, reported a hospital-acquired infection rate of 4%. They used one nurse epidemiologist in a 1200-bed institution and surveyed only hospital "acquired" infections. Our ongoing studies, however, have continued to show significant rates since we have increased our surveillance team to three nurse epidemiologists in our 840-bed institution.

McNamara, *et al.*[2] in a two month incidence study reported a hospital "ac-

quired" infection rate of 6%. In their study, only infections cultured and recorded in the hospital bacteriology laboratory were reported. Relevant to this is the fact that 23.4% of our patients with infections on the medical and surgical services did not have cultures taken when indicated. Sixty-nine percent of this group were patients with hospital onset infections (Table VI).

Edwards[6] at the Staten Island United States Public Health Service Hospital noted a community onset infection rate of 17.9% and a hospital onset infection rate of 7.8% in a one-month incidence study. The patient population involved was predominantly adult male "world travelers" and thus, differs considerably from our patient population. In the same study, 13.5% of patients received prophylactic antimicrobials and 21.0% received therapeutic antimicrobials, which is lower than our rates of 28.3% and 31.0% respectively (Tables XII and XIII).

Our community onset infection rate was 16.9%. Our hospital onset infection rate of 12.9% was higher than any of the above quoted figures, except those from Boston City Hospital (Table X). Comprehensive surveillance, compromised hosts, prolific use of antimicrobials, and extensive surgical procedures and method of study all contribute to these high rates.

Table VII shows a predominance of gram negative bacteria, 75.6% among the total bacterial isolates. This is in keeping with recent reports from several different institutions.

We believe that the low rates of hospital onset infections seen in newborns (less than 1%) and obstetrics (8%) was more apparent than real and was related in part to a short hospital stay on these services, without post-discharge followup (Table IX). Some hospital onset infections on the surgical and medical services are probably missed for similar reasons.

The data presented here indicate that the nurse-epidemiologists performed well in their ability to identify and classify infections (Table II).

On the medical and surgical services, they picked up 11 patients not on antimicrobials who had infections which the physicians did not find (Table III). This occurred primarily because the physicians did not use the "procedure index," which lists incidental procedures such as bladder or wound irrigations performed on the patients by the nursing staff as a screening device, whereas the nurses did. Since the 57 infected patients discovered by the physicians and not by the nurses had the same general distribution as all infected patients surveyed (Tables II and III), they were most likely missed by the nurses because of their less frequent visits to the ward (once per week as contrasted with two or three times per week by the physicians). Both physicians and nurses agreed on the classification of 335 patients without infections, and 152 patients with infections. The nurses correctly classified 36 patients with infection whom the physicians judged not to have infection. Accordingly, the nurses were incorrect in 36/534 of patients or 6.7% of the time. We believe that medical and surgical epidemiologic visits should be made two times per week, and pediatric and obstetric areas visited three times per week because of their more rapid turnover. Based on our experience, relatively complete surveillance of all infections and antimicrobial use requires one nurse-epidemiologist for every 250 to 300 hospital beds.

It would appear that these encouraging results justify our belief in the ability of the properly trained nurse epidemiologist to collect data acoording to standardized criteria. The high rate of antimicrobial use and hospital-associated infections points out the necessity for ongoing programs of this type. We believe that this method is accurate enough so that major trends can be determined and possible control methods evaluated.

Since this study, our surveillance team was increased to three nurses who surveyed the entire hospital, and we have subsequently utilized a digital computer for storage of the data, with monthly reports available to all interested sections of the hospital.

SUMMARY

A twelve-day period-prevalence study of hospital-associated infections and antimicrobial use was carried out by three physicians and two nurse-epidemiologists from the infectious disease section. Data collected by the nurses was compared in detail with that obtained by the physicians. Using this mode of comparison, the reliability of the nurses in collecting such data was substantiated; their error rate was only 6.7%.

The total hospital onset infection rate was 12.9% of patients, and the community onset infection rate was 16.9% of patients. The hospital-associated infection rate was 28.9%.

The hospital-associated infection rate on the medical and surgical services was 37.2%. The community onset infection rate was 19.3%, and the hospital onset infection rate was 19.4% on these two services. On the same services 23.4% of patients did not have cultures taken when indicated; 31% of patients received therapeutic antimicrobials; and 28.3% received prophylactic antimicrobials during their hospital stay. Hospital-associated infections were found in 8% of obstetric patients, 26.3% of pediatric patients, and 0.8% of newborn patients.

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COMPUTERIZATION OF A CLINICAL BIOCHEMISTRY LABORATORY

Howard H. Sky-Peck
Max Rafelson, Jr.

INTRODUCTION AND GENERAL OPERATION OF THE SYSTEM

In recent years there has been an ever-increasing demand for the performance of hospital laboratory services. In biochemistry laboratories this demand has been met, for the most part, by the introduction of various automated techniques to replace manual performance of the tests. These automated techniques have been successful in performing the tests, but they have made the existing data-handling operations of the laboratory inadequate and outmoded. Thus, the technical ability to perform the tests exceeded the ability to sort and catalogue the specimens and requisitions, calculate the results, and return coherent, usable data promptly to the patient's chart.

Our Clinical Biochemistry Laboratory will process an average of 450 specimens per day and will perform some 800,000 biochemical tests per year. In an attempt to solve the pressing problems of data acquisition and processing, we began in October, 1967 to computerize our laboratory operation. At that time we decided, and in fact still believe, that the only rational and reliable way to proceed was to employ small *dedicated* computers for the tasks of real-time test data acquisition and processing in the laboratory. The essential tasks of a laboratory data processing system are to accept requests for tests, to prepare these test requests for the technican and/or automated instrument involved, to gather and verify the results of completed tests, and to report these results in a coherent form to the consumer. We selected the *Berkeley Scientific Laboratory* (*BSL*) system for our laboratory. This laboratory-based system includes both hardware and software and is designed to perform all the essential tasks indicated above and thus relieves the technician of considerable clerical and data processing functions. This real-time, time-sharing system, which has undergone considerable evolution since 1967, consists at present of the following:

(1) A PDP 8/L computer with 4K 12 bit words of core, a 360,448-word fixed-head disc, and a 12 channel I/O multiplexor. This is the central processor of the *BSL Clindata System* and can handle a Technicon SMA 6/60, two teletypes, a Kleinschmidt printer, a 1200 lines per minute printer, an IBM-compatible magnetic tape, and a peripheral processor (to be discussed below). We will add very shortly an additional magnetic tape and 4K

From the Department of Biochemistry, Rush Medical College, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Howard H. Sky-Peck, Ph.D., Professor and Acting Chairman, Department of Biochemistry
Max E. Rafelson, Jr., Ph.D., Professor, Department of Biochemistry and Associate Dean for Biological and Behavioral Sciences and Services

words of core to allow off-line statistical analyses of test performance and to generate chronological summaries of patient data.

(2) The peripheral processor (*BSL Chemdata System*) is an on-line, real-time computer system (PDP 8/L) that simultaneously monitors a number of single and dual channel Technicon AutoAnalyzers. With the 4K core of the PDP 8/L the system capacity is 12 channels of Auto Analyzers and 12 different tests. The *Chemdata System* is a subsystem of the *BSL Clindata Laboratory System*. Loading sheets which prescribe the sequence of standards and patient specimens in the AutoAnalyzer sampler tray are generated upon request. Final test results are automatically calculated and transferred to the Clindata disc where they remain in a limbo area until the technologist verifies their acceptability and releases them by command to the patient files. We shall discuss the operation of this subsystem in more detail later.

OPERATION OF THE CENTRAL PROCESSOR (BSL CLINDATA)

In our institution the specimens and requisitions arrive simultaneously at the laboratory with no forewarning. The specimen is assigned a 4-digit accession number, and patient information and test requests by code number are entered onto the computer disc via teletype. This process requires only several seconds per patient entry and may be done simultaneously on two teletypes. The question and answer dialogue between the teletype operators and the central processor is shown in Figure 1, in which that portion printed automatically by the program is underlined. This entry segment asks sequentially for the specimen (accession) number, the patient's unique 7-digit hospital number, the patient's name, the attending physician's name, the location of the patient, as well as for any specific information (message), and for the tests requested. Following entry of the numerical test codes (1,2,3, etc.) the program prints back the test code numbers (001.002, 003, etc.) and the test names (sodium, potassium, chloride, etc.) and asks for verification (OK?). If verified, the operator's answer is Y(ES) and the program asks for the next specimen number until this program segment is exited from by appropriate command. If the tests are not verified, the operator's answer is N(O) and the program will automatically ask for tests again. In the first example shown in Figure 1, 19G is simply a group code that calls in both the total and direct bilirubin. Other group codes are available to request the four electrolytes or the four electrolytes plus glucose and urea nitrogen.

Following entry of patient information and test requests onto the disc, a number of useful listings or queries may be generated on command at any time. These include:

(1) *Patient Directory* (Figure 2)
This is an alphabetic listing of all patients and includes their location in the hospital and their unique 7-digit hospital number. This shows laboratory personnel precisely which patient specimens have arrived in the laboratory. Other listings of this type are possible, such as listing by attending physician and listing by ascending hospital number.

(2) *Master Work Sheet* (Figure 3)
This lists, by ascending specimen number, all tests to be performed on each specimen. In conjunction with the directory, it is thus a simple matter to determine whether or not a given test was requested on a given patient specimen.

(3) *Worksheet by Individual Test* (Figure 4)
This is a listing by individual tests of specimens on which that particular test has been requested and serves as a worksheet for the technician performing that test.

(4) *Incomplete Worksheet* (Figure 5)
This is a listing of tests not completed on a specimen by ascending specimen number. This allows laboratory personnel to be aware of the status of the daily work load at any time.

(5) The status of tests on any patient may be ascertained at any time by a simple query command. When all tests are completed on a specimen, a laboratory report is generated automatically.

(6) There are a number of other useful program segments which include among others:

(A) A carry-over command that transfers to the next day all incomplete work so that it is not overlooked.

```
*E
SPEC. #0031
MED REC. # 0000000
NAME GIRAFFE PAMELA
DR. ROSOFF A
LOCATION ZOO OP
MSG. 11AM

TESTS 1 2 3 4 7 8 9 5 12 14 19G 20 25 28
 001  SODIUM
 002  POTASSIUM
 003  CHLORIDE
 004  CO2 CONTENT
 007  GLUCOSE
 008  UREA NITROGEN
 009  CREATININE
 005  CALCIUM
 012  SGOT
 014  LDH
 019  BILIRUBIN, TOTAL
 098  BILIRUBIN, DIRECT
 020  ALK PHOSPHATASE
 025  CHOLESTEROL, TOTAL
 028  URIC ACID

 OK? Y

SPDC. #0609
MED REC. # 1738367
NAME ALLEN HELEN
DR. THOMSON A
LOCATION 321
MSG. STAT

TESTS 1 2 3 4 32
 001  SODIUM
 002  POTASSIUM
 003  CHLORIDE
 004  CO2 CONTENT
 032  MAGNESIUM

 OK? Y
```

Fig. 1—Patient Entry Teletype Dialogue

NAME	SPEC#	LOCATION	MED REC#	06/19/70
AA BLANK	0006		0000000	
AHRENS ELEANOR	0623	603	1934572	
ALLEN HELEN	0609	321	1738367	
@NDERSON WALTER	0026	619	1765173	
@NDERSON,IRZNE	0283	MSHC	3833561	
ANDERSON,W	0020	10 E	1765173	
ATKINS EUGENE	0615	1118	1941694	
ATKINS,E	0016	1118	1941694	
AVERY,LILLIE MAE	0288	MSHC	3036169	
BAKER,MARY	0301	CLINIC	0017892	
BAKER4MARY	0660	MED-3	1017892	
BALLA HENRY	0611	1118	1746819	
BALLENTINE IB	0600	7JNUR	1941197	
BARKER INEZ	0028	330	1758406	
BARKER,INEZ	0323	330	1758406	
BOONE ALLIE	0178	374	1879750	
BRUNO SALV@TORE	0608	309	1800859	
BRYSON JAMES	0167	1028	1940198	
BYRNE JOHN	0147	250	1941527	
BYRNE,JOHN	0303	250	1941527	
CHAMBERS VINA	0065	820	1937540	
CHOL CONTROL ELEV	0005		0000000	
CHOL CONTROL	0004		0000000	
COLEMAN,BEATRICE	0666	1136B	1939758	
CONTROL A	0001		0000000	

Fig. 2—Alphabetic Patient Directory

(B) A numerical count of each individual type of test performed each day.

(C) A patient billing and accounting summary report.

Figure 6 is an example of a patient's report. This patient, a tall slender female, was selected because we did not believe she would object to the public disclosure of her blood chemistry values. Her name is Pamela and she is a giraffe from one of our local zoos. The values to the right are normal human values in our laboratory. We cannot at this time comment as to whether or not Pamela's blood values are normal, although we can envy her cholesterol and uric acid levels. Three copies of the report are generated simultaneously on the line printer without

MASTER WORKSHEET. 06/19/70

0600 BALLENTINE IB..... 019 098

0601 JENKINS IG..... 005 007 019 098

0602 MUELLER CLARENCE.....-032

0603 KUDLA DOROTHY.....-028

0604 PEREZ IG..... 019 098

0605 JARRETT IB..... 019 098

0606 PETER VLASTA..... 001 002 003 004-005

0607 JUSZKIEWICZ ALEX..... 001 002 003 004

0608 BRUNO SALVATORE..... 001 002 003 004

0609 ALLEN HELEN..... 001 002 003 004-032

0610 KOWALCZWK SOPHIE..... 001 002 003 004 007 008 009 012 014
019 098 020

Fig. 3—Master Worksheet

```
WORKSHEET:  TEST #032--MAGNESIUM                    TIME 1155  06/23/70

 0013    ........    WHITCOMB, EDISENE          913

 0018    ........    WHITCOMB,E                 913

 0045    ........    LANZARATTA PHILIP          286

 0049    ........    CHIARO MARIE               416

 0112    ........    MORRISON PAULINE           370

 0260    ........    LANDOR GREGORIA            275

 0348    ........    CRUZEN IMOGENE             259

 0468    ........    BARACH,STEVE               382

 0614    ........    RISER ELIZABETH            926

 0615    ........    SMITH OSCAR                603
```

Fig. 4—Worksheet by Individual Test

```
INCOMPLETE WORKSHEET.                                   06/19/70

 0602  MUELLER CLARENCE.........-032

 0603  KUDLA DOROTHY............-028

 0606  PETER VLASTA.............-005

 0609  ALLEN HELEN..............-032

 0611  BALLA HENRY..............-001-002-003-004-007-008

 0612  DAWSON RAMONA............-001-002-008

 0613  HOBBS VERA...............-001-002-008

 0614  HAYNES ELIZABETH.........-001-002-008

 0618  HOOD GENERAL.............-004-007-008-011

 0619  DUNLAP MYRTLE............-001-002-003-004-007-008

 0620  NELSON ROSE..............-001-002-003-004-007-008-024

 0621  SMITH OSCAR..............-001-002-003-004-007-008-018
```

Fig. 5—Incomplete Worksheet

benefit of carbon. The bond original on top is the medical chart copy. The second copy is on action paper and is the attending physician's copy. The third action sheet is the laboratory copy.

What is of interest in this report is that the data arrived to the final endpoint, the patient report, via several pathways. The Na, K, Cl, CO_2, glucose, and urea nitrogen tests were done on the Technicon SMA 6/60. Cholesterol, uric acid, and creatinine results were obtained automatically from single channel AutoAnalyzers monitored by the *Chemdata System* and were, after verification, released to the specific patient file. The remainder of the data were obtained by manual or semi-automated methods not interfaced

*0000000 GIRAFFE PAMELA DR.: ROSOFF A ZOO OP
DATE OF ENTRY: 06/19/70 11AM SPEC #0031

001 SODIUM	144	MEQ/L	137-148
002 POTASSIUM	4.0	MEQ/L	3.4-5.2
003 CHLORIDE	99	MEQ/L	98-110
004 CO2 CONTENT	25.0	MEQ/L	21-29
007 GLUCOSE	97	MG%	70-115
008 UREA NITROGEN	23	MG%	8-22
009 CREATININE	2.3	MG%	0.65-1.1
005 CALCIUM	9.6	MG%	8.5-10.8
012 SGOT	29	UNITS	10-35
014 LDH	680	UNITS	200-400
019 BILIRUBIN, TOTAL	1.4	MG%	0.3-1.0
098 BILIRUBIN, DIRECT	.3	MG%	0.1-0.4
020 ALK PHOSPHATASE	1.1	UNITS	0.6-2.5 ADULT
025 CHOLESTEROL, TOTAL	35	MG%	140-280
028 URIC ACID	.5	MG%	3.0-6.8

Fig. 6—Patient Laboratory Report

with the computer. Results were sent to the computer manually via a data-input console. Figure 7 shows a BSL general purpose data-input console. To send data, the technologist keys in the 3-digit test number (upper left field), a 4-digit specimen number (upper right field), and the appropriate test results (lower field). Depressing the SEND key transmits the data to the computer disc patient file.

Any results residing in the disc memory can be overridden with corrected data, and a new print-out is automatically generated. In addition the console will inform the technologist immediately of a test not requested, of a specimen number not on file, or redundant data (a result is already present for the test), or that results are out of limits, or that there are no data to override. The technologist can query the computer from the console for

Fig. 7—General Purpose Data-Input Console

the result of any test on any patient and have it displayed instantaneously in the console data field. Up to 128 separate messages may be composed and assigned a code number and can be sent from the console in place of numerical data. Examples of the comment capabilities are:

111: Report to follow.
001: Specimen hemolyzed—please redraw.
005: Specimen not received.
013: No urine received to complete tolerance.

OPERATION OF PERIPHERAL PROCESSOR (BSL CHEMDATA)

The peripheral processor (PDP 8/L) samples each AutoAnalyzer recorder once per second and determines the peak height in percent transmission. This transmission value is then converted to digital concentration units using linear interpolation from a set of calibration standards associated with each run. A special multiplexor analog-digital converter was developed for this purpose and is used in conjunction with re-transmitting potentiameters attached to the strip chart recorders. A correction is applied for drift encountered in the analyzer system. The processor detects and notifies the technologist, by print-out, of aberrant conditions such as abnormal drift, missing peaks, and abnormally high peaks as they may occur during the run. As noted previously, the data are stored in a limbo area on the disc until verified and released to the patient file by the technologist.

The purpose of the Chemdata processor is to free the technologist from the unproductive tasks of calculating results and "baby-sitting" the AutoAnalyzers since the computer independently monitors the output of the analyzers and notifies the user of the occurrence of malfunctions. Human errors are removed from peak-height determination and calculation of concentration values. The results with the Chemdata are more consistent because the laboratory director establishes the criteria for acceptability of results and the computer tirelessly and faithfully monitors the system, reporting any inconsistencies. Deteriorations in the AutoAnalyzer equipment such as worn tubing and small holes in the dialyzers are predicted by Chemdata.

The peak determinator judges the validity of peaks based upon both shaping and timing criteria. The program automatically enters time synchronization after encountering the first peak. From that point, the program is searching for a peak during the open period of a floating time aperture. A *peak* is defined as the maximum portion of the curve that must spend a specified minimum number of seconds at approximately zero slope. It is also required that the slope of the curve be sufficiently positive when the aperture opens and sufficiently negative and below the peak when the aperture closes. When the aperture closes, the program checks the peak timing orientation and makes an appropriate shift if time synchronization is changing.

In practical operation, the technologist asks via command teletype for an AutoAnalyzer loading list which prescribes the sequence of standards and patient specimens to be placed in the AutoAnalyzer sampler tray. Such an original loading list is shown in Figure 8. The first plate of the run contains the system's calibration standards followed by unknown specimens in specified groups separated by a drift correction standard and other suitable control specimens. For successive plates the same option may be used or more usually the first plates calibration standards are used with a drift standard every ten specimens. (Figure 9).

A small *BSL analyzer console* located next to each AutoAnalyzer provides the technologist with a convenient link to coordinate his actions with those of the AutoAnalyzer and the computer which is located in a remote area. Whenever the sampler is started or stopped the peripheral processor must be so informed. The basic commands used to operate the program are: START, DONE, PAUSE, CONTINUE, and RELEASE. The START command informs the system

```
TEST GROUPS: 3
(M)ED. REC.#, (N)AME, (U)NIT, (S)TATUS: NS
FROM TRAY# 1
LOADING TRAY SHEET   TEST GROUP: 3      1120
009  CREATININE


01-01  STDS
01-02  STDS
01-03  STDS
01-04  STDS
01-05  STDS
01-06  STDS
01-07  STDS
01-08  ISTD
01-09  DSTD
01-10  0001  CONTROL A                  -009
01-11  0002  CONTROL B                  -009
01-12  0003  CONTROL C                  -009
01-13  0006  AA BLANK                   -009
01-14  0042  CARUSO LENA               -009
01-15  0071  PITZELE NATHANIEL         -009
01-16  0076  CARUSO LENA               -009
01-17  0077  NASSMAN LOUISE            -009
01-18  0078  MITERA LORETTA            -009
01-19  DSTD
01-20  0082  MATHIS MARCIE             -009
01-21  0083  TYSSEN TONY               -009
01-22  0084  ZIFF CLARENCE            -009
01-23  0090  CHALMERS JUANTIA          -009
01-24  0091  CHALMERS JUANITA          -009
01-25  0092  CHEWS NELS                -009
01-26  0093  WIECK MARY                -009
01-27  0094  SCHMIDT BEVERLY           -009
01-28  0096  DOWELL ESTHER             -009
01-29  DSTD
01-30  0098  BROWN ROSE                -009
01-31  0100  ELY HARRIET               -009
01-32  0101  LYNN HARRY                -009
01-33  0103  CORMAN ERSKINE            -009
01-34  0104  DANIELSON REUBEN          -009
01-35  0107  PAULSEN WILLIAN           -009
01-36  0109  CEH JOSEPH                -009
01-37  0110  RAHSER JOHN               -009
01-38  0116  NELSON MARGARET           -009
01-39  DSTD
01-40  DSTD
LOADING TRAY SHEET   TEST GROUP: 3      1120
009  CREATININE
```

Fig. 8—Initial Loading Tray List for AutoAnalyzer

that a run is to begin with calibration standards. The program enters a search mode to find the first peak and achieve time synchronization. The DONE command specifies the specimen at which the run will terminate. Operation may be temporarily suspended by the PAUSE command in case some technical problems arise. The CONTINUE command is used when the sampler is restarted and requires only one drift correction standard to re-enter time synchronization. The RELEASE command informs the Clindata program that data stored in a limbo area on the disc can be released to the patient file.

02-01	DSTD		
02-02	CNRL		
02-03	0117	PETER VLASTA	-009
02-04	0118	BRUNO SALVATORE	-009
02-05	0131	COLEMAN ELIZBETH	-009
02-06	0137	HAMPER LOUISE	-009
02-07	0139	THOMASON WALTER	-009
02-08	0141	GARCIA PATRICIA	-009
02-09	0161	CAMPBELL ELIZABETH	-009
02-10	0170	BUILTA STEVEN	-009
02-11	DSTD		
02-12	0174	FELLS JOE	-009
02-13	0180	KARR DAVID	-009
02-14			
02-15			
02-16			
02-17			
02-18			
02-19			
02-20			
02-21	DSTD		
02-22			
02-23			
02-24			
02-25			
02-26			
02-27			
02-28			
02-29			
02-30			
02-31	DSTD		
02-32			
02-33			
02-34			
02-35			
02-36			
02-37			
02-38			
02-39			
02-40	DSTD		

Fig. 9—Continuation Loading Tray List for AutoAnalyzer

Positive operator verification messages are provided to the user by the Chemdata processor. All commands are confirmed by print-out on a local teletype in the analyzer area. The tray-cup number, test name, and percent transmission for each calibration and drift standard encountered is printed by the teletype. This enables the technician to verify whether the system is in synchronization or if some problem has developed. If a sampler's response does not satisfy the criteria of a peak, it is flagged as an error. Figures 10 and 11 show some of the types of data provided by the system. *Error code 1* is an error diagnostic message indicating that the no peak was found. In this case the apparent error was due to a saline (blank) having been placed in this position. *Error code 8* indicates the

```
TEST-GROUP   4 CHOL
START =   0

STD CHOL
CHOL              1- 1 ..S.. 74.6
CHOL              1- 2 ..S.. 59.4
CHOL              1- 3 ..S.. 46.2
CHOL              1- 4 ..S.. 36.2
CHOL              1- 5 ..S.. 22.4
CHOL              1- 6 ..S.. 12.8
CHOL              1- 7 ..S.. 34.4


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CHEMISTRY REPORT
TEST-GROUP   4 CHOL


                PLATE
                   CUP CODE VALUE (MG%)
                 1- 8 ..S.. 205.
                 1- 9 ..... 160.
                 1-10 ..... 257.
                 1-11 ..... 370.
                 1-12 ..... 180.
                 1-13 ..... 329.
                 1-14 ..X..   1
                 1-15 ..... 181.
                 1-16 ..... 204.
                 1-17 ..... 144.
                 1-18 ..S.. 210.
```

Fig. 10—ChemData Print-out

specimen has exceeded the highest standard in the calibration curve and must be rerun after suitable dilution. Other types of errors such as system errors or unacceptable drift of base line are also indicated. The major advantages this system has over human endeavor is its ability to search in the time domain, its constancy of decision, and its lack of human fatigue.

CONCLUSIONS

A few concluding remarks on the total BSL system are in order. Perhaps two of the more important aspects of any laboratory computer system are its reliability and its ease of operation. The reliability of the present system has been extremely good and only minor hardware problems have arisen. Some of the

CHEMISTRY REPORT
TEST-GROUP 4 CREATININE

PLATE CUP	CODE	VALUE (MG%)
2-21	..S..	2.0
2-22	.....	4.3
2-23	..X..	1
2-24	.....	.6
2-25	.....	1.8
2-26	..X..	8
2-27	.....	3.9
2-28	.....	1.8
2-29	.....	1.8
2-30	.....	1.2
2-31	..S..	2.1

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CHEMISTRY REPORT
TEST-GROUP 1 URIC ACID

PLATE CUP	CODE	VALUE (MG%)
1-28	..S..	6.5
1-29	.....	5.1
1-30	.....	5.8
1-31	.....	5.7
1-32	.....	3.4
1-33	.....	11.5
1-34	.....	5.6
1-35	.....	5.1
1-36	.....	9.2
1-37	.....	2.6
1-38	..S..	6.4

Fig. 11—ChemData Print-out

components are more reliable than others, and as might be expected, the mechanical components are less reliable. The two computers have been essentially trouble-free. With regard to the second point, the system is operated totally and effectively by our laboratory personnel. We have not hired engineers or programmers or in fact any specialized individuals to run the system. The computer system operates 24 hours per day, seven days a week.

The soft-ware package is most reasonable at this stage of its evolution. There are some restrictions in the time-sharing operating system and in the structure of some of the files which limit the flexibility of the system. Significant changes are expected shortly which should materially improve the system in terms of its flexibility for the individual user. We are interfacing our system with a larger computer located elsewhere in the institution which can deliver our data to remote stations in the hospital, a process which is best *not* done by a real-time laboratory system. A system identical with ours is being installed in the hematology laboratories and presumably will also be interfaced with the larger computer. This will serve to set in operation some of the elements of a total hospital laboratory information system.

It is our considered opinion after some 30 months of experience that the BSL system has materially aided our laboratory. It has been possible for us to accommodate a markedly increased work-load without hiring additional personnel. Quality has improved and the time required to deliver a much more coherent report to the patient's chart has decreased. A non-trivial benefit to the laboratory is that we now know which specimens arrived in the laboratory, what was requested, and what the status of any specimen is at any time—in other words, what did we get and what have we done about it?

We should point out that computerizing a laboratory is not achieved without some trauma and frustrations. Everything that is done by a busy laboratory still must be done in addition to installing new systems and procedures, training ourselves and other personnel in our laboratory, and eliciting the cooperation and understanding of personnel in many other areas of the hospital. We have a singular debt to the personnel of the Department of Biochemistry for their splendid cooperation and determination, and above all for their unfailing patience.

LETTER TO THE EDITOR

Sir:

In a previous report[1] we described the operation of the Family Planning Clinic in the Presbyterian-St. Luke's Health Center. In that report we presented the statistics accruing after a two-year operation of that clinic.

These figures can now be brought up to date with a report on the success of this program over a four-year period:

Total number of patients enrolled	3,496
Total number of patient visits	11,970
Patients active after four years	2,282
This gives us a "dropout" rate of	34.5%

BREAKDOWN BY METHOD

	Oral	IUCD	Diaphragm	Foam
No. of patients	2,116	1,100	140	140
No. of visits	6,119	3,425	215	186
No. of "dropouts"	745	360	26	42
% of "dropouts"	25.2%	35.2%	40.7%	30.0%

Combined "dropout" rate = 34.5%

Since a 50% "dropout" rate is generally reported over a one to two year period, our figures can be considered as rather spectacular.

ARTHUR H. KLAWANS, M.D.

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1. Klawans, AH & Mayer, JC: The Family Planning Service. Presbyterian-St. Luke's Hospital Medical Bulletin 7:147, Oct., 1968